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TITLE: Selective Cytotoxic Phospholipids for Prostate Cancer

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CONTRACTING ORGANIZATION: The University of Tennessee
Memphis, TN 38163

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13. ABSTRACT (Maximum 200 Words)

The goal of this project is to build upon our discovery of two phospholipid lead compounds, serine amide phosphate (**SAP**) and serine diamide phosphate (**SDAP**), that have been shown to be selective in their cytotoxic actions in PC-3 and DU-145 prostate caner cells respectively. These agents were originally desingned as part of a series of compounds to inhibit lysophosphatidic acid (LPA), a phospholid growth factor. After discovering the antiproliferation activity of SAP and SDAP in prostate cancer cell lines we propose to snythesize a focused set of SAP and SDAP analogs. We have found that the synthesis of these compounds can be prepared in a shorter sequence and in better yield using our new sythetic scheme. We have tested for the affinity of the synthesized compounds in PC-3, DU-145, and LNCaP cell lines as we proposed earlier. In addition to these cell lines we have also tested for affinity of these compounds in two additional PPC-1 and TSU cell lines (data shown in Table 1). These new analogs have provided valuable insight as to the importance of chirality, lipid solubility, spatial orientation, and important functional groups of the pharamcophore and for the optimization of the antiproliferative actions of this new set of drugs. Our most recent compounds are based on the thiazolidinones (**2**) and the thiazolidine (**3**) analogs. We have utilized new synthetic schemes for these new compounds and have found the optimum length of the aliphatic chain in these two series. In earliar studies it appeared in our Serine Amide Phosphate (SAP) series that the alphatic chain is optimum at C-14 while with the new compounds it apprears to be C-18 on DU-145 and PC-3 cell lines. In a few instances we have discovered a new set of 2-arylthiazolidine-4-carboxylic acid amides that show sub micromolar anticancer activity in the cell lines described above. We have designated this set of compouns as 2-arylthiazolidine-4-caroxylic acid amides (ATCAAs). This reports shares the critical structure activity relationships for optimum acitivity in prostate cancer cells.

14. SUBJECT TERMS
-arylthiazolidine-4-carboxylic acid amides, cytotoxic, nirality, pharmacophore, lipid solubility, synthesis,

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68

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Table of Contents

Introduction The goal of this project is to build upon our discovery of two phospholipid lead compounds, serine amide phosphate **(SAP)** and serine diamide phosphate **(SDAP)**, that have been shown to be selective in their cytotoxic actions in PC-3 and DU-145 prostate caner cells respectively. These agents were originally desingned as part of a series of compounds to inhibit lysophosphatidic acid (LPA), a phospholid growth factor. After discovering the antiproliferation activity of SAP and SDAP in prostate cancer cell lines we propose to snythesize a focused set of SAP and SDAP analogs using the combinatorial parallel-compound solution phase synthesies when appropiate, and to prepare the remaining analogs using classical techniques. These analogs provided us with valuable insight as to the importance of chirality, lipid solubility, spatial orientation, and important functional groups of the pharamcophore and allow for the optimization of the antiproliferative actions of this set of drugs.

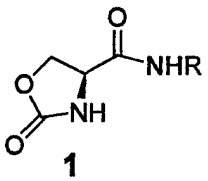
More recently we have discovered a new set of 2-arylthiazolidine-4-carboxylic acid amides that show sub micromolar anticancer activity in the cell lines described above. We have found new synthetic schemes for these new compounds and have expanded our structure activity relationships into the substitutions for activity against PC-3, DU-145, LNCaP, PPC-1 and TSU-Pr1 prostate cell lines using the RH7777 cell line as a control cell line for comparison. We are now optimizing these agents for potential use in prostate cancer.

Due to time and budgetary constraints, only a limited set of compounds including will be carried forward to these studies in the next year of the grant. These experiments are designed to provide an initial pharmacologic assessment of our most promising compounds, focusing specifically on (1) their in vivo toxicity and (2) their in vivo antitumor efficacy in prostate tumor xenografts. Animal care guidelines at our institution will be strictly followed for these studies. We have requested a year no cost extension to carry out these study and optimizing 2-arylthiazolidine-4-caroxylic acid amides (ATCAAs) for prostate cancer.

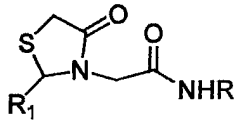
Task 1. Synthesis of serine amide phosphate (SAP) and serine diamide phosphate (SDAP) analogs

Year 3: We will take the advantage of biological studies in year 1 and 2 to design new generation of analogs of SAP and SDAP in order to optimize the inhibition of proliferation of prostate cancer cells.

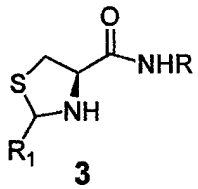
This task was successfully completed. In year 2 we proposed the design and synthesis of a new series of conformationally restricted analogs represented by compounds **1**, **2** and **3** as shown below. The heterocyclic scaffold (oxazolidine, thiazolidinone or thiazolidine) serves as a nonhydrolyzable metabolically stable phosphate mimic. To test this hypothesis we have synthesized a series of above-mentioned analogs and evaluated for their ability to inhibit the growth of five human prostate cancer cell lines. Oxazolidine analog **1** was less cytotoxic in all five prostate cancer cell lines. Compared to oxazolidine derivative, thiazolidinones were more potent but were less cytotoxic than SAPs. Interestingly, thiazolidinones (**2**) demonstrated improved selectivity against non-tumor RH7777 cells. The most potent and selective compounds were obtained with the thiazolidine scaffold. With these very encouraging preliminary results, to further optimize the thiazolidine pharmacophore in terms of cytotoxicity and selectivity, we proposed the design, synthesis and biological evaluation of several new thiazolidine analogs in year 3 and the details are described in this report. ^{1}HNMR, ^{13}CNMR, IR and mass spectrometry confirmed the structures of all new compounds synthesized.



1



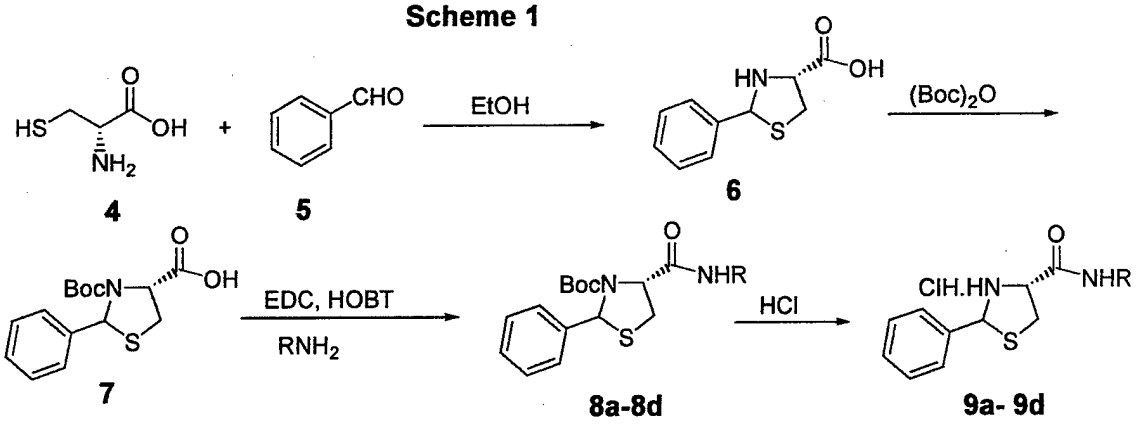
2



3

Examination of the cytotoxic effects of **9a-9c** (Scheme 1) showed that as the chain length increased from C_7 to C_{18}, the potency also increased. However, a further increase in the alkyl chain length by one carbon unit (**9d**) caused a significant loss of activity. Interestingly, the C_{14} derivative (**9b**) demonstrated higher potency than **9a**, but was 8-fold less selective against the RH7777 cell line. Thus, an alkyl chain with C_{18} unit was optimal for maintaining the potency and selectivity observed in this series of compounds.

Scheme 1

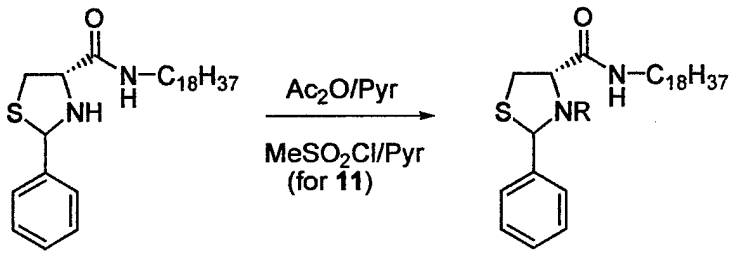


R

a C_7H_{15}
b $C_{14}H_{29}$
c $C_{18}H_{37}$
d $C_{19}H_{39}$

To understand the effect of derivatization of the secondary amine functionality compounds **10** and **11** were synthesized as shown in Scheme 2.

Scheme 2

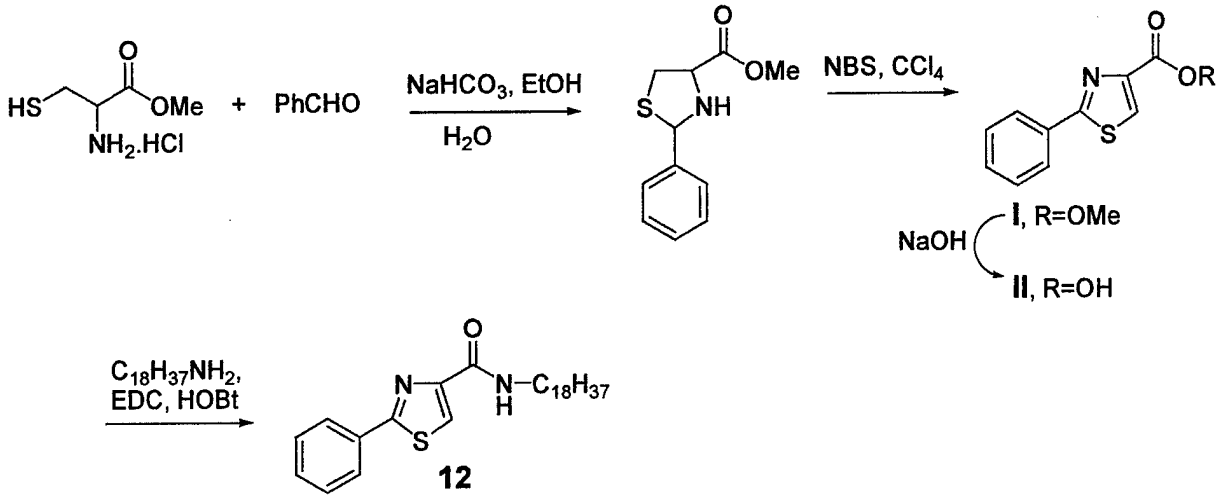


10, R=$COCH_3$
11, R=SO_2CH_3

To understand the effect of unsaturation on potency and selectivity, and to overcome the problems associated with stereoisomers, we replaced the central thiazolidine core in **9c** with a thiazole ring (Scheme 3). However, thiazole derivative (**12**) did not show any

activity below 20 μM in both prostate and RH7777 cells, which suggests that thiazolidine ring with two chiral centers plays an important role in providing potency and selectivity.

Scheme 3



12

Replacements of the phenyl ring in **9c** with a heterocycle, such as an indole, pyridine or furan ring was investigated by synthesizing analogs **17-19**. Introduction of a methylene spacer separating the phenyl ring and the thiazolidine ring furnished a compound **16** as shown below. Replacement of the phenyl ring in **9c** with an alkyl or cyclohexyl group afforded compounds **14** and **15**. The synthesis and cytotoxicity data of compounds **20-34** provides a summary of a broad survey of phenyl ring substituted analogs. All these compounds were synthesized as shown in Scheme 4.

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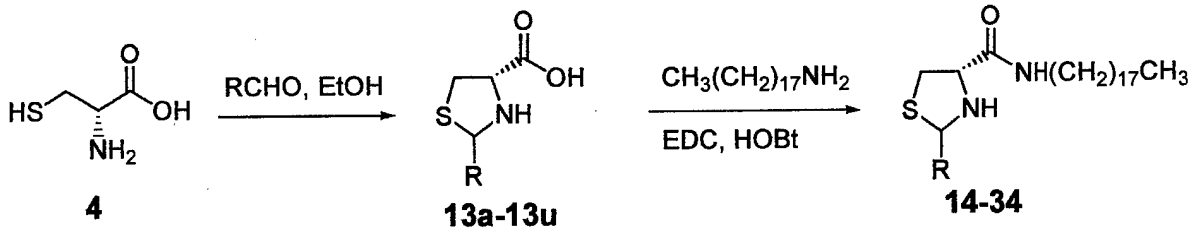
The University of Tennessee

Scheme 4



4 **13a-13u** **14-34**

Compd	R
13a	n-dodecyl
13b	cycohexyl
13c	benzyl
13d	3-indolyl
13e	3-pyridinyl
13f	3-furanyl
13g	4-dimethyl amino phenyl
13h	3-hydroxyphenyl
13i	4-methoxyphenyl
13j	3,4-dimethoxyphenyl
13k	3,4,5-trimethoxyphenyl
13l	4-acetylamino phenyl
13m	4-fluorophenyl
13n	4-bromophenyl
13o	4-nitrophenyl
13p	4-cyanophenyl
13q	3,5-difluorophenyl
13r	2,6-dichlorophenyl
13s	3-bromo-4-fluorophenyl
13t	4-methylphenyl
13u	biphenyl

Task 2. Determine activity of SAP and SDAP analogs in Prostate cell lines

Year 3: We will determine the activity of the synthesized analogs in Specific Aims 1.2.3.4.5.6 in PC-3, DU-145 and LNCaP cell lines.

This task was completed successfully. We have tested the cytotoxicity of the synthesized compounds in PC-3, DU-145 and LNCaP prostate cancer cell lines as proposed earlier. In addition to these cell lines we have also tested in two additional PPC-1 and TSU-Pr1 prostate cancer cell lines. To determine the selectivity of these compounds we have also tested them in non-prostate cancer cells RH7777 cells (data shown in Table1)

Task 3. Determine the activity of SAP and SDAP analogs in prostate tumor xenograft in mice

Year 3: We will select the most promising agents from specific Aim.6 of the PC-3, DU-145 and LNCaP cell lines studied in year 1, 2 and 3 for In Vitro Efficacy against Prostate Tumor Xenografts in mice (Specific Aim C.7)

Starting with SAPs, we identified a series of novel and cytotoxic thiazolidine amides based on a 4-thiazoldine carboxylic acid scaffold. Among this series, we synthesized and carried out a detailed SAR studies and evaluated their antiproliferative activity against five human prostate cancer cell lines and RH7777 cells (negative controls). This data (Table 1) suggests that introduction of ring activating groups on the phenyl ring resulted in increasing potencies for prostate cancer cell lines leading to discovery of several new anticancer agents with low/sub micromolar cytoxicity and high selectivity. Based on the results of this cytotoxicity study we chose four compounds (**9b**, **9c**, **24** and **25**) for the xenograft studies in mice. We have completed the synthesis of gram quantities of these four analogs and in vivo studies are in progress in our laboratory.

Key Research Accomplishments

- Identified 2-Aryl-thiazolidine-4-carboxylic acid amides (ATCAAs) as a new class of cytotoxic agents for prostate cancer.
- Carried out systematic and detailed SAR of ATCAAs by synthesizing a large number of derivatives and evaluated them for their inhibitory activity towards the growth of five human prostate cancer cell lines.
- Observed that derivatization of amine functionality in ATCAAs to the corresponding amides or sulfonamides results in loss of biological activity.
- Observed that replacement of the 2-phenyl ring in ATCAAs by an alkyl or cyclohexyl groups and introduction of a methylene spacer separating the phenyl ring and the thiazolidine ring reduced the potency.
- Investigated the effect of various substitutions on the 2-phenyl ring on cytotoxicty and selectivity. Introduction of electron-donating groups on the phenyl ring resulted in increasing potencies with enhanced selectivity, leading to discovery of several new anticancer agents with low/sub micromolar cytotoxicity.

Reportable Outcomes (copies attached)

- Discovery of 2-Arylthiazolidine-4-carboxylic acid amides as a New Class of Cytotoxic Agents for Prostate Cancer
  Veeresa Gududuru, Eunju Hurh, James Dalton, and Duane Miller. 227th ACS National Meeting, Anaheim, CA, March 28-April 1, 2004.
- 2-Phenylthiazolidine Derivatives of Lysophosphatidic Acid as a New Class of Anticancer Agents
  Eunju Hurh, Veeresa Gududuru, Duane Miller and James Dalton. 95th Annual AACR Meeting, Orlando, FL, March 27-31, 2004.
- Synthesis and Antiproliferative Activity of 2-Aryl-4-oxo-thiazolidin-3-yl-amides for Prostate Cancer
  Veeresa Gududuru, Eunju Hurh, James Dalton and Duane Miller. 31st Annual MALTO Meeting, Memphis, TN, May 16-18, 2004.
- Synthesis and biological evaluation of novel cytotoxic phospholipids for prostate cancer
  Veeresa Gududuru, Eunju Hurh, Gangadhar Durgam, Seoung Soo Hong, Vineet Sardar, Huiping Xu, James T. Dalton, Duane D. Miller, *Bioorg. Med. Chem. Lett.* 2004, 14, 4919-4923.
- Synthesis and antiproliferative activity of 2-Aryl-4-oxo-thiazolidin-3-yl amides for prostate cancer
  Veeresa Gududuru, Eunju Hurh, James Dalton, and Duane Miller, *Bioorg. Med. Chem. Lett.* 2004, 14, 5289-5293.
- Efficient Microwave Enhanced Synthesis of 4-Thiazolidinones
  Veeresa Gududuru, Viet Nguyen, James T. Dalton and Duane D. Miller, *Synlett.* 2004, (in press).
- Discovery of 2-Arylthiazolidine-4-carboxylic acid amides as a New Class of Cytotoxic Agents for Prostate Cancer
  Veeresa Gududuru, Eunju Hurh, James Dalton, and Duane Miller, *J. Med. Chem.* 2004 (submitted).

Conclusions

We have discovered a new set of 2-arylthiazolidine-4-carboxylic acid amides (ATCAAs). This reports shares the critical structure activity relationships (SAR) for optimum acitivity in prostate cancer cells. We have shown sub micromolar anticancer activity in the PC-3, DU-145, LnCaP, PPC-1 and TSU-Pr1 prostate cell lines using the RH7777 cell line as a control cell line for comparison (see Table 1). We have found new synthetic schemes for these new compounds and have expanded our structure activity relationships into the substitutions patterns for optimum anti-prostate cancer activity. We are now investigating the action of several of these newly found anti-prostate cancer agents *in vivo*.

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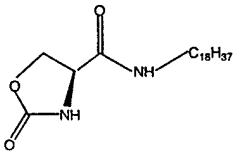
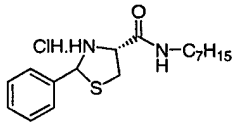
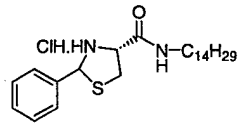
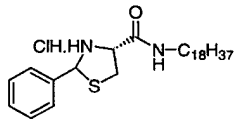
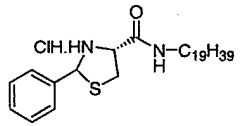
Table 1

Compound ID	Structure	IC_{50} (μM)					
		RH7777	DU145	PC-3	LNCaP	PPC-1	TSU-Pr1
1		35.2	22.1	17.2	31.3	11.6	27.7
9a		52.2	44.9	38.5	12.4	34.7	28.0
9b		3.4	2.4	3.0	1.4	1.3	2.0
9c		25.6	5.4	7.8	2.1	2.0	5.0
9d		No activity	> 20	No activity	13.6	16.8	> 20

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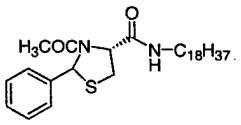
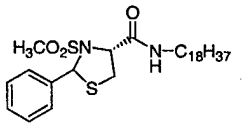
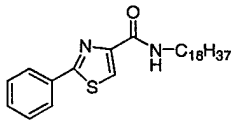
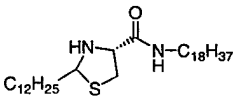
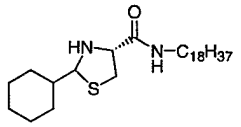
Compound ID	Structure	IC_{50} (μM)					
		RH7777	DU145	PC-3	LNCaP	PPC-1	TSU-Pr1
10		>20	~20	~20	16.1	12.6	>20
11		>20	>20	>20	>20	>20	>20
12		>20	>20	>20	>20	>20	>20
14		~20	8.9	15.0	11.9	13.0	10.7
15		> 20	> 20	> 20	12.8	9.3	> 20

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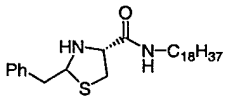
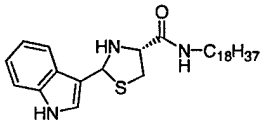
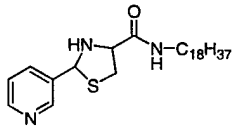
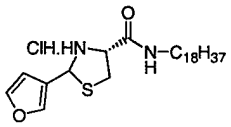
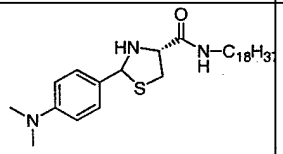
Compound ID	Structure	IC_{50} (μM)					
		RH7777	DU145	PC-3	LNCaP	PPC-1	TSU-Pr1
16		> 20	15.3	16.4	4.4	4.0	11.2
17		> 20	8.9	11.5	2.1	1.3	4.4
18		10.5	7.5	9.2	3.6	2.9	7.8
19		10.4	6.6	8.1	1.7	1.1	4.2
20		> 20	5.3	6.0	1.6	1.1	3.0

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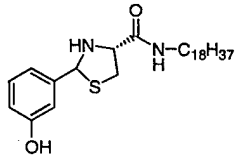
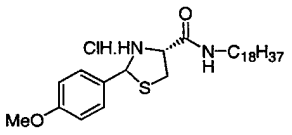
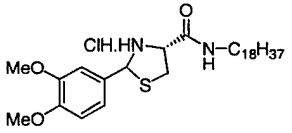
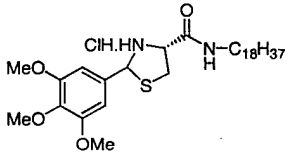
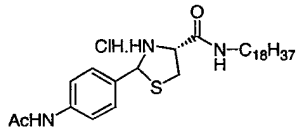
Compound ID	Structure	IC_{50} (μM)					
		RH7777	DU145	PC-3	LNCaP	PPC-1	TSU-Pr1
21		31.0	5.7	6.7	1.7	1.2	4.0
22		>20	8.7	~20	2.1	1.5	ND
23		10.3	4.5	5.2	0.85	0.58	2.4
24		11.4	3.9	4.0	0.82	0.48	2.4
25		21.1	3.1	5.6	1.3	0.55	0.94

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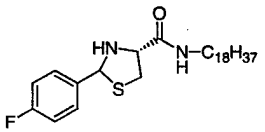
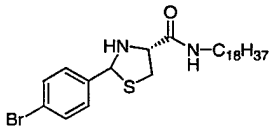
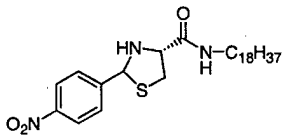
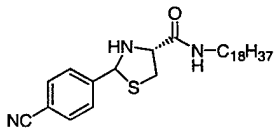
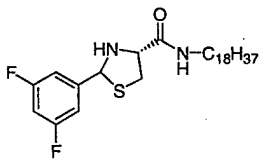
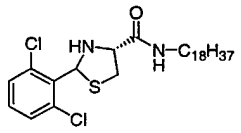
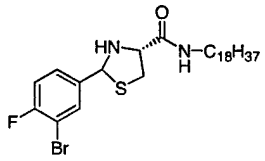
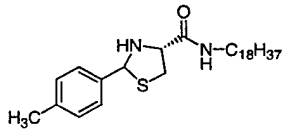
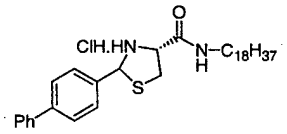
Compound ID	Structure	IC_{50} (μM)					
		RH7777	DU145	PC-3	LNCaP	PPC-1	TSU-Pr1
26		17.4	5.7	6.8	1.9	2.1	5.4
27		> 20	13.8	17.3	5.1	3.7	18.3
28		~ 20	15.3	~ 20	8.4	15.3	15.9
29		>20	>20	>20	5.9	5.0	>20
30		>20	>20	>20	11.2	10.6	>20

P.I. Duane D.Miller Ph.D.
The University of Tennessee

Compound ID	Structure	IC_{50} (μM)					
		RH7777	DU145	PC-3	LNCaP	PPC-1	TSU-Pr1
31		> 20	> 20	> 20	13.1	17.1	~ 20
32		~ 20	11.3	13.5	3.0	4.7	14.0
33		> 20	10.5	12.8	1.9	1.9	8.0
34		>20	>20	>20	>20	>20	>20

Discovery of 2-Arylthiazolidine-4-carboxylic acid amides as a New Class of Cytotoxic Agents for Prostate Cancer

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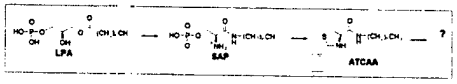
Abstract

Prostate cancer is the single most common cancer in American males and is the second leading cause of cancer deaths in men in the United States. None of the conventional approaches to cancer therapy have proven successful for prostate cancer. It is also known that certain treatments-radiation therapy, radical prostatectomy or hormonal therapies can have detrimental effects on urinary, bowel, and sexual functions. Thus, development of a safe chemotherapeutic agent without any side effects would be an attractive proposal for the treatment of prostate cancer. We recently identified a novel series of cytotoxic serine amide phosphates (SAPs) for prostate cancer. These compounds are very effective in killing specific prostate cancer cell lines with IC_{50} values ranging from about 2 μM to 50 μM. However, in spite of their high cytotoxicity they are not selective in differentiating cancer cells and healthy cells. It is also evident that phosphate group in this class of compounds is susceptible for hydrolysis by lipid phosphatases. To improve the selectivity, cytotoxicity and metabolic stability, we made some closely related structural changes on SAPs, which led us to discovery of a new class of 2-arylthiazolidine-4-carboxylic acid amides with IC_{50} values at low micromolar range and enhanced selectivity. This new series of compounds were evaluated in PC-3, DU 145, LNCaP, PPC-1, and TSU-Pr1 human prostate cancer cell lines for their cytotoxicity. The details of synthesis, structure-activity relationship (SAR), and results of biological studies will be presented in this presentation

Introduction

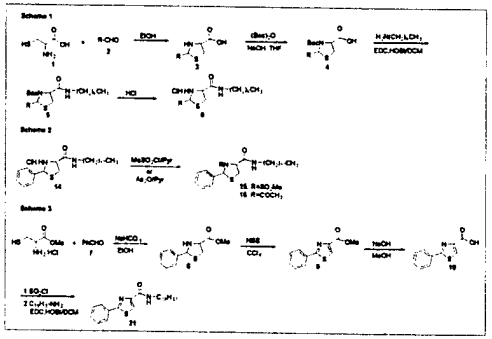
G-protein coupled receptors are a family of membrane-bound proteins that are involved in the proliferation and survival of prostate cancer cells initiated by binding of lysophospho-lipids (LPL).[1-4] The importance of G protein-dependent pathways in the regulation of growth and metastasis *in vivo* is corroborated by the observation that the growth of androgen-independent prostate cancer cells in mice is attenuated by treatment with pertussis toxin, an inhibitor of Gi/o proteins.[5] Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) is a lipid mediator generated via the regulated breakdown of membrane phospholipids that is known to stimulate GPCR-signaling. Prostate cancer represents a unique disease in which to exploit the activity of these LPL mimetics and antagonists. Advanced prostate cancers express LPL receptors [6]and depend on phosphatidylinositol 3-kinase (PI3K) signaling for growth and progression to androgen independence.[7] Thus, these pathways are widely viewed as one of the most promising new approaches to cancer therapy, [7] and provide an especially novel approach to the treatment of advanced, androgen-refractory prostate cancer. Despite the promise of this approach, there are no clinically available therapies that selectively exploit or inhibit LPL or PI3K signaling. Our laboratory is interested in development of LPA agonists and antagonists. As part of this study, we recently identified a novel series of serine amide phosphates [8] (SAPs) that are effective in killing specific prostate cancer cell lines (Figure 1). Although many of these compounds showed low micromolar cytotoxicity, they were non-selective (i.e., they potently killed the prostate and control cell lines). We hypothesized that biomimetic replacement of the easily hydrolysable, metabolically unstable, and poorly penetrating phosphate group in our lead compound with a thiazolidine pharmacophore would enhance the physicochemical, pharmacokinetic, and antiproliferative properties and result in highly potent and selective anticancer agents for prostate cancer. To this end, we modified our initial approach and designed a new series of 2-arylthiazolidine-4-carboxlic acid amides (ATCAAs) in which the 2-arylthiazolidine moiety was introduced as a phosphate mimic (Figure 1)

Figure 1



Chemistry

Commercially available cysteine (R or S form) was allowed to react with various aldehydes in EtOH to give acid 3, which was converted to corresponding N-Boc derivative (4). Reaction of N-Boc-2-arylthiazolidine-4-carboxylic acid (4) with various alkyl amines under standard coupling conditions using EDC and HOBt gave corresponding amides 5. Treatment of these amides with HCl gave the target compounds in high yield (Scheme 1). N-Sulfonyl and N-acyl derivatives (15 & 16) were synthesized from 14 as shown in Scheme 2. For the preparation of unsaturated thiazole derivative, cysteine was converted to thiazole carboxylic acid (10), which was coupled with octadecyl amine to give 21 as shown in Scheme 3



Biological Methods

We examined the cytotoxicity of thiazolidine derivatives in five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, TSU-Pr1) and in a negative control cell line (RH7777) that lacks LPL receptor using the sulforhodamine B (SRB) assay. Cells were exposed to a wide range of concentrations (0 to 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 cytotoxicity we tested 5-fluorouracil (5-FU) against all five prostate cancer cell lines. The results are summarized in Table 1.

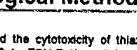
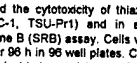
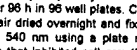
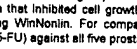
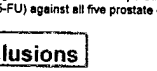
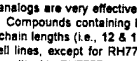
Conclusions

Thiazolidine analogs are very effective in killing prostate cancer cell lines with IC_{50} values ranging from about 1 μM to over 100 μM. Compounds containing long alkyl chains (i.e., 14) were more potent and selective than derivatives with shorter alkyl chain lengths (i.e., 12 & 13). Stereoisomers (14 & 17) showed a difference in their cytotoxicity in all of the four tumor cell lines, except for RH7777, which suggests a stereospecific interaction with a molecular target that is absent or less critical in RH7777 cells. N-Sulfonyl and N-acyl derivatives (15 & 16) were less cytotoxic compared to the free amine (14). Fully unsaturated thiazole analog (21) was less potent than the parent thiazolidine compound (14). Replacement of the 2-aryl group of 14 with an alkyl or heteroaryl group resulted in loss of biological activity. We observed that several of the thiazolidine analogs were as potent inhibitors of tumor cell proliferation as 5-FU, and were measurably better in many cell lines. Compared to SAPs, ATCAAs in which the 2-arylthiazolidine moiety was introduced as a phosphate mimic showed selective anti-prostate cancer activity in all of the tumor and negative control cell lines. Further studies will focus on optimization of the alkyl chain length, investigation of the importance of chirality and understanding of other structural requirements of the thiazolidine analogs for enhanced selective cytotoxicity in prostate cancer cell lines.

Acknowledgements

This work is supported by a grant from the Department of Defense (DAMD17-01-1-0830).

Table 1. Structures and Antiproliferative Activities of ATCAAs in Prostate Cancer Cell Lines (IC_{50} in μM)

ID	Structure	All[illegible]	DU 145	PC-3	LNCaP	PPC-1	TSU-Pr1
SAP1		ND	10.8	~20	2.6	1.6	11.1
SAA2		2.5	3.2	2.4	3.3	1.6	1.1
11		>100	>100	>100	>100	>100	>100
12		52.2	44.9	38.5	12.4	34.7	28.0
13		3.4	2.4	3.0	1.4	1.3	2.0
14		25.6	5.4	7.8	2.1	2.0	5.0
15		>20	>20	>20	>20	>20	>20
16		>20	~20	~20	16.1	12.6	~20
17		19.1	~1	>10	6.3	4.0	~10
18		>20	8.7	~20	2.1	1.5	ND
19		>20	>20	>20	11.2	10.6	ND
20		>20	>20	>20	5.9	5.0	ND
21		>20	>20	>20	>20	>20	ND
22		>20	>20	>20	>20	>20	>20
23		~20	8.9	15.0	11.9	13.0	10.7
24		10.5	7.5	9.2	3.6	2.9	7.8
5-FU		ND	11.9	12.0	4.9	6.4	3.6

References

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6. Murillo, H. Huang, H. Schmidt, L. J. Smith, D. I. Tindall, D. J, *Role of PI3K signaling in survival and progression of LNCaP prostate cancer cells to the androgen refractory state.* Endocrinology, 2001. 142(11): p. 4795-805.
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8. Miller, D. D. T., Gabor. Dalton, James T. Sardar, Vineet M. Elrod, Don B. Xu, Huiping. Baker, Daniel L. Wang, Dean. Liliom, Karoly. Fischer, David J. Virag, Tamas. Nusser, Nora U.S. Patent. *Ser. No. 611,638* 2003

2-Phenylthiazolidine Derivatives of Lysophosphatidic Acid as a New Class of Anticancer Agents

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[2]Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee, Memphis, TN 38163

Abstract

Lysophosphatidic acid (LPA) is a growth factor that promotes cell proliferation, cell survival, tumor cell invasion, and metastasis via activation of G-protein coupled receptors. A series of LPA analogs with a 2-phenylthiazolidine ring, a bioisostere of the phosphate moiety, were synthesized as individual stereoisomers, and tested for their growth inhibitory activity in prostate cancer cell lines. Cytotoxicity of the compounds was determined by the sulforhodamine B assay in 96-well plates in PC-3, LNCaP, DU 145, PPC-1, and TSU-Pr1 human prostate cancer cells exposed to increasing concentrations of the compounds for 96 hours. IC_{50} values obtained by nonlinear regression analysis ranged from 1 μM to about 50 μM. The length of the alkyl chain attached to the phenylthiazolidine ring via an amide bond had a significant influence on cytotoxicity. Compounds with a 14-carbon chain (C14) were the most potent, followed by C18 and C7. LPA-nonresponsive RH7777 rat hepatoma cells were employed as a control cell line to discriminate LPA receptor-mediated effects from nonspecific cytotoxicity of the analogs. A compound with a C18 chain demonstrated selective activity against prostate cancer cells (IC_{50} of 2 μM to 8 μM) when compared to RH7777 cells (IC_{50} of 26 μM). In addition, the (R) isomer of this compound showed approximately 2-fold higher activity than the (S) isomer against prostate cancer cells. Further, no stereoselective effect was observed in RH7777 cells. The (R) isomer at 3 μM induced apoptosis in LNCaP cells. We also examined the effect of these compounds on AKT phosphorylation status using immunoblot analysis. Treatment of LNCaP cells with a representative compound at 10 μM resulted in complete loss of phospho-AKT within 6 hours. Additional studies to identify and verify molecular target(s) of these compounds are ongoing in our laboratory.

Keywords: cytotoxicity; prostate cancer; apoptosis

Introduction

Various therapies for advanced prostate cancer, including chemotherapy, have not proven satisfactory. One promising drug development strategy for prostate cancer involves identifying and testing agents that interfere with growth factors and other molecules participating in signaling pathways. LPA and sphingosine 1-phosphate are phospholipid growth factors that elicit their effects (i.e. stimulation of cell proliferation and tumor cell invasion) via multiple subtypes of membrane spanning G-protein coupled receptors (GPCRs), collectively referred to as lysophospholipid receptors. Among downstream mediators of these GPCRs, phosphoinositide 3-kinase (PI3K) and AKT proteins are two major stimulators of cell survival and cell proliferation. Therefore, structural modifications of lysophospholipids to antagonize/inhibit molecules in this signaling pathway will result in reduced cell proliferation and survival. Our laboratory recently identified and tested a novel series of serine amide phosphate derivatives for their growth inhibitory activity in prostate cancer cells. To improve metabolic stability, pharmacokinetic properties, and cellular penetration, a thiazolidine moiety was introduced in place of the phosphate group of these serine amide phosphates. As further efforts to examine structure-activity relationships and to identify molecular target(s) of these new series of thiazolidine analogs, the present studies examined their growth inhibitory activities, apoptotic activity, and effect on AKT phosphorylation status in prostate cancer cell lines (DU 145, PC-3, LNCaP, PPC-1, and TSU-Pr1).

Methods

❑ Cell Culture and Cytotoxicity Assay (Sulforhodamine B assay)

Cancer cell lines were obtained from American Type Culture Collection (Manasses, VA). Prostate cancer cells and RH7777 cells were maintained in RPMI 1640 and DMEM medium containing 2 mM L-glutamine supplemented with 10% fetal bovine serum and maintained in 5% CO_2 / 95% air humidified atmosphere at 37 °C. Cells were plated in 96-well plates, allowed to attach overnight, and incubated with 200 μL of growth media containing a wide range of an analog for 96 hours. At the end of the drug treatment, the media were removed, and the cells were fixed with 10% trichloroacetic acid at 4 °C for several hours before washing 5 times with water. The plates were air dried overnight. Next, fixed cells were stained with 0.4% (w/v) SRB solution for 20 minutes at room temperature. After staining, SRB solution was decanted, and plates were quickly rinsed 5 times with 1% acetic acid to remove unbound dye and air dried overnight. The cellular protein-bound SRB was then dissolved in 10 mM Tris base (pH 10.5) for 40 minutes on a rocking platform shaker, and absorbance at 540 nm was measured using a plate reader. Percentage of cell survival was calculated by absorbance at 540 nm in compound-treated cells divided by absorbance in untreated control cells. Percentages of cell survival versus drug concentrations were plotted and the concentration of drug that inhibited cell growth by 50% (IC_{50}) was determined by nonlinear regression using WinNonlin (Pharsight Corporation, Mountain View, CA).

❑ Apoptosis Assay

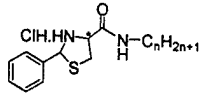
DNA fragmentation by agarose gel electrophoresis: LNCaP cells were treated with a thiazolidine derivative for 24 to 108 hours. Total DNA was extracted from 2 x 10^6 cells by simple centrifugation method, treated with RNase and Proteinase K. After precipication in ethanol, DNA was reconstituted in Tris-EDTA buffer, separated on agarose gels, and visualized by ethidium bromide staining.

Quantification of DNA fragmentation by Cell Death Detection ELISA: A sandwich ELISA (Roche, Mannheim, Germany) utilizing monoclonal antibodies specific for DNA and histones was used to quantify degree of apoptosis induced by the analogs after 72 hour exposure to a compound. This assay measures DNA-histone complexes (mono- and oligonucleosomes) released into cytoplasm from the nucleus during apoptosis. RH7777 cells were employed because of nonspecific cytotoxicity of compound 2 in receptor-negative RH7777 cells as well as receptor-positive prostate cancer cells.

❑ AKT Inhibition

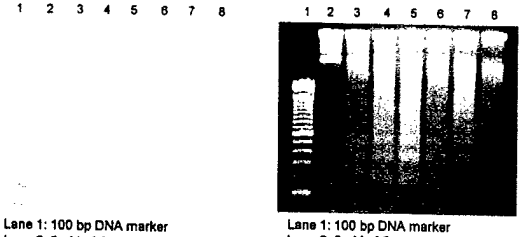
30 μg of total cellular protein from untreated control cells and compound-treated cells were separated by SDS-PAGE, transferred to nitrocellulose membrane, and total AKT and phospho-AKT were probed with anti-AKT and anti-phospho AKT antibody specific for AKT phosphorylated at Ser 473, respectively (Cell Signaling Technology, Beverly, MA). The immunoblots were visualized by enhanced chemiluminescence, and changes of relative levels of phospho-AKT compared to total AKT by analog treatment were quantified by densitometric analysis.

Results



Cell Line	Compound	1	2	3	4
	n	7	14	18	18
	Isomer	(R)	(R)	(R)	(S)
DU 145	IC_{50} (μM)	44.9	2.4	5.4	7.1
PC-3		38.5	3.0	7.8	> 10
LNCaP		12.4	1.4	2.1	6.3
PPC-1		34.7	1.3	2.0	4.0
TSU-Pr1		28.0	2.0	5.0	~ 10
RH7777		52.2	3.4	25.6	19.1

LNCaP cells

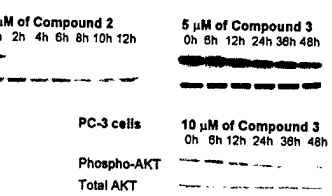


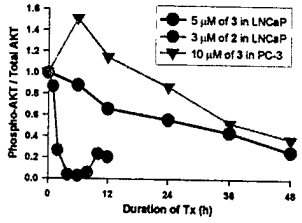
Lane 1: 100 bp DNA marker
Lane 2: 5 μM of Compound 2 for 36 h
Lane 3: 3 μM of Compound 2 for 24 h
Lane 4: 3 μM of Compound 2 for 24 h
Lane 5: 3 μM of Compound 2 for 48 h
Lane 6: 3 μM of Compound 2 for 72 h
Lane 7: 3 μM of Compound 2 for 108 h

Lane 1: 100 bp DNA marker
Lane 2: 5 μM of Compound 3 for 24 h
Lane 3: 5 μM of Compound 3 for 48 h
Lane 4: 5 μM of Compound 3 for 72 h
Lane 5: 5 μM of Compound 3 for 96 h
Lane 6: 3 μM of Compound 3 for 96 h
Lane 7: 8 μM of Compound 3 for 48 h
Lane 8: 8 μM of Compound 3 for 72 h

$$\text{Enhancement Factor} = \frac{OD_{405\,nm} \text{ in Treated Cells}}{OD_{405\,nm} \text{ in Control Cells}}$$

Cell Line	Compound 2			Compound 3			Compound 4		
	2 μM	5 μM	10 μM	2 μM	5 μM	10 μM	2 μM	5 μM	10 μM
PC-3	1.8	18.7	54.0	1.4	2.3	3.4	1.1	1.2	1.4
LNCaP	14.1	75.4	80.7	4.5	46.2	37.1	3.1	5.4	25.8
RH7777	2.6	3.2	2.5	Not determined			Not determined		





Conclusions

❑ Compound **1** with a C_7 alkyl chain was generally inactive showing IC_{50} > 10 μM in all the cell lines tested.

❑ Compound **2** with a C_{14} alkyl chain was the most potent inducer of apoptosis and inhibitor of tumor cell growth. Apoptotic activity of this compound was selective in prostate cancer cells despite nonselective cytotoxicity in RH7777 negative control cells. Compound **2** at 3 μM completely inhibited AKT phosphorylation after 4 hour treatment, correlating with high cytotoxity.

❑ Compound **3** with a C_{18} alkyl chain demonstrated selective cytotoxicity against prostate cancer cells (IC_{50} of 2 μM to 8 μM) without any growth inhibitory effect up to 20 μM in receptor-deficient RH7777 cells. It also induced apoptosis in a dose-dependent manner, and inhibited AKT phosphorylation without affecting AKT protein expression levels.

❑ Compound **4** was less potent in inhibiting prostate cancer cell growth than the R isomer (compound **3**), suggesting the importance of stereochemistry for antiproliferative effects. Stereoselective cytotoxicity was also observed in apoptotic activity with compound **3** being more potent inducer of apoptosis at all the concentrations tested.

❑ Thiazolidine analogs of lysophospholipids represent a novel class of potential anticancer agents in prostate cancer. Further studies will focus on correlating the cytotoxic effects of the analogs with their roles in lysophospholipid receptor signaling, identifying molecular target(s) of the analogs, and understanding structural requirements of the analogs for selective cytotoxicity.

Acknowledgement

This work is supported by a grant from the USAMRMC (#PC010431).

Synthesis and Antiproliferative Activity of 2-Aryl-4-oxo-thiazolidin-3-yl amides for Prostate Cancer

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Abstract

arious therapies for advanced prostate cancer, including chemotherapy, have not roven satisfactory. One promising drug development strategy for prostate cancer nvolves identifying and testing agents that interfere with growth factors and other olecules participating in signaling pathways. LPA (Lysophosphatidic acid) and phingosine 1-phosphate are phospholipid growth factors that elicit their effects (i.e. timulation of cell proliferation and tumor cell invasion) via multiple subtypes of embrane spanning G-protein coupled receptors (GPCRs), collectively referred to as sophospholipid receptors. Among downstream mediators of these GPCRs, hosphoinositide 3-kinase (PI3K) and AKT proteins are two major stimulators of cell urvival and cell proliferation. Therefore, structural modifications of lysophospholipids antagonize/inhibit molecules in this signaling pathway will result in reduced cell roliferation and survival. Our laboratory recently identified and tested a novel series f serine amide phosphates (SAPs) for their growth inhibitory activity in prostate ancer cells. To improve metabolic stability, pharmacokinetic properties, and cellular enetration, a thiazolidinone moiety was introduced in place of the phosphate group of ese SAPs, which led us to discovery of a new class of 2-aryl-4-oxo-thiazolidin-3-yl nides. This new series of compounds were evaluated in PC-3, DU 145, LNCaP, PC-1, and TSU-Pr1 human prostate cancer cell lines for their cytotoxicity. The details synthesis, structure-activity relationship (SAR), and results of biological studies will presented in this presentation

Introduction

rine amide phosphates[1,2] (SAPs) were identified as a new series of compounds that fectively kill specific prostate cancer cell lines and represent a novel class of ticancer agents for the treatment of prostate cancer (Figure 1). We made odifications to SAPs in the phosphate head group region and this group was replaced a thiazolidinone moiety toward improving pharmacokinetic properties and cellular tivity in human prostate cancer cell lines.

idres[3] et al have postulated that 4-thiazolidinones may be recognized as diphosphate mics, and using this scaffold they synthesized and evaluated some 4-thiazolidinones their ability to inhibit the bacterial enzyme MurB. Based on this hypothesis, we signed a new series of thiazolidinone derivatives as shown in Figure 2. In the present rk, we report the synthesis, structure-activity relationship (SAR) studies and the tiproliferative activity of type I compounds (Figure 2), in five human prostate cancer ll lines.

Figure 1

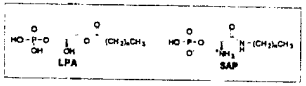
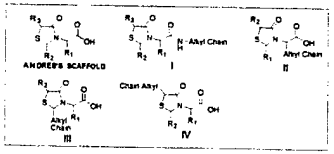
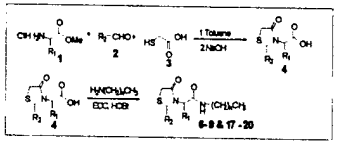


Figure 2

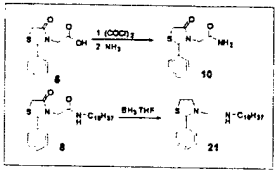


Chemistry

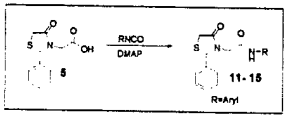
Scheme 1



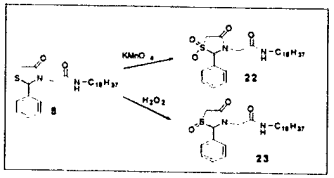
Scheme 2



Scheme 3



Scheme 4



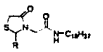
Biological Methods

We examined the cytotoxicity of thiazolidinone derivatives in five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, TSU-Pr1) and in a negative control cell line (RH7777) that lacks LPL receptor using the sulforhodamine B (SRB) assay[4]. Cells were exposed to a wide range of concentrations (0 to 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[5]. For comparative purposes and to understand the degree of cytotoxicity we tested 5-fluorouracil (5-FU) against all five prostate cancer cell lines. The results are summarized in Table 1- 3.

Table 1 (IC_{50} in μM)

Compound	R	RH7777	DU145	PC-3	LNCaP	PPC1	TSU-Pr1
5	OH	NA	>50	>50	>50	>50	>50
6	NHC_6H_{13}	20	22.4	20.3	14.1	15.8	19.7
7	NHC_8H_{17}	16.4	19.6	13.5	14.1	10.1	13.4
8	$NHC_{18}H_{37}$	39.6	12.6	11.1	9.3	7.1	8.5
9	$NHCH_2CN$	>100	>100	>100	>100	>100	>100
10	NH_2	>100	>100	>100	>100	>100	>100
11		70.9	69.0	74.1	24.1	46.2	53.2
12		25.4	16.2	18.1	14.5	13.1	16.1
13		34.9	24.0	28.6	13.2	20.5	17.2
14		>100	>100	>100	82.5	>100	60.8
15		>100	>100	>100	31.4	>100	69.9
16		>100	>100	>100	>100	>100	>100
5-FU		ND	11.9	12.0	4.9	6.4	3.6

Table 2 (IC_{50} in μM)



Compound	R	RH7777	DU145	PC-3	LNCaP	PPC1	TSU-Pr1
17		>50	>50	>50	>50	>50	>50
18		>50	>50	>50	>50	>50	>50
19		31.1	14.8	12.6	11.8	10.7	17.5
20		>50	>50	>50	>50	>50	>50

Table 3 (IC_{50} in μM)

Compound	Structure	RH7777	DU145	PC-3	LNCaP	PPC1	TSU-Pr1
21		>20	15.8	>20	>20	12.0	6.1
22		22.1	15.5	8.5	10.9	5.5	9.3
23		11.5	11.2	6.5	7.9	5.4	6.4

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Acknowledgements

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Summary

- Biomimetic replacement of the phosphate group in SAPs by hydrolytically more stable thiazolidinone scaffold offered a novel series of cytotoxic agents for prostate cancer.
- These compounds showed an alkyl chain length dependent cytotoxicity. As the chain length increases from 10C to 18C (compounds 6,7 & 8) the cytotoxicity also increases.
- Removal of the lipophilic alkyl chain or replacement by hydrophobic aryl groups decreases the biological activity.
- 2-Aryl-ring substitution and functionality/size are sensitive to cytotoxicity.
- Sulfur oxidation is tolerated as compounds 22 & 23 showed good cytotoxicity.

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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

Keywords: Prostate cancer; LPA; New agents; Cytotoxicity.
☆ Part of this work was presented at 225th ACS National Meeting, New Orleans, LA, March 23–27, 2003 and at 94th Annual AACR Meeting, Washington, DC, July 11–14, 2003.
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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

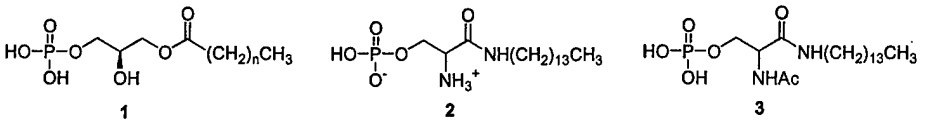
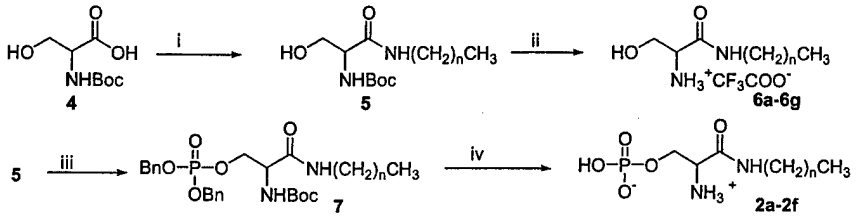
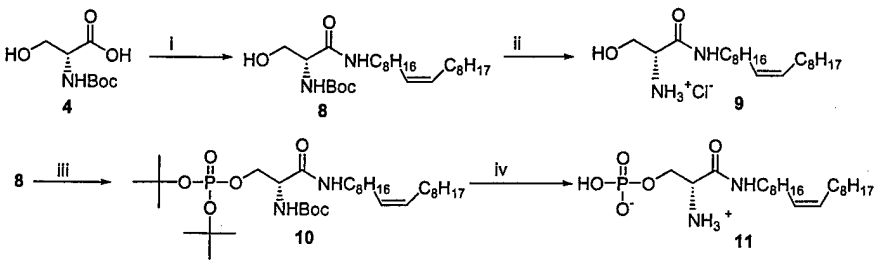


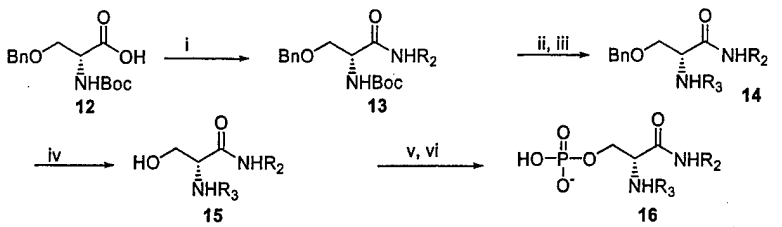
Figure 1. Structures of LPA and SAPs.



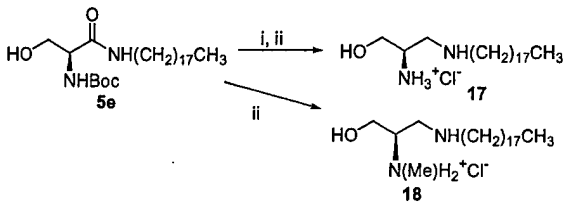
Scheme 1. Reagents and conditions: (i) $CH_3(CH_2)_nNH_2$, EDC, HOBt, CH_2Cl_2, rt, 5 h; (ii) TFA, CH_2Cl_2, rt, 0.5 h; (iii) tetrazole, dibenzyl diisopropylphosphoramidite, CH_2Cl_2, rt, 0.5 h, H_2O_2, rt, 0.5 h; (iv) H_2, 10% Pd/C, EtOH, rt, 3 h.



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.

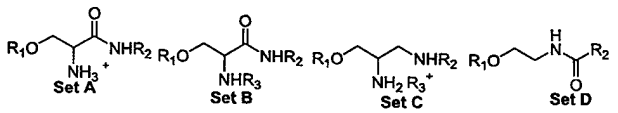
When the amide group in serine amide alcohols was reduced to produce long chain *N*-alkyl amino alcohols **17** and **18**, these analogues retained cytotoxicity and were very effective in killing prostate cancer cell lines with low micromolar cytotoxicity. To determine the selectivity, several of synthesized 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

This research was supported by a Grant from the Department of Defense (DAMD17-01-1-083).

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



Set	Compound (chirality)	R_1	R_2	R_3	IC_{50} (μM)						
					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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Synthesis and antiproliferative activity of 2-aryl-4-oxo-thiazolidin-3-yl-amides for prostate cancer☆

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Abstract—We have previously described serine amide phosphates (SAPs) as a novel class of cytotoxic agents for prostate cancer. Several of them showed potent cytotoxicity against human prostate cancer cell lines, but were not selective in non-tumor cells. To improve the selectivity and further enhance the potency, we designed a new series of 2-aryl-4-oxo-thiazolidin-3-yl amides. The current work describes synthesis, SAR, and biological evaluation of these compounds for their ability to inhibit the growth of prostate cancer cells. The antiproliferative effects of synthesized compounds were examined in five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, and TSU), and in RH7777 cells (negative controls). From this study, three potent compounds (**8**, **20**, and **21**) have been detected, which are effective in killing prostate cancer cells with improved selectivity compared to SAPs.

Prostate cancer accounts for 33% of all newly diagnosed malignancies among men in the United States.[1] According to the American Cancer Society,[2] an estimated 230,110 men will be diagnosed with prostate cancer in 2004, and 29,900 men will die of it. The incidence of prostate cancer varies worldwide, with the highest rates found in the United States, Canada, and Scandinavia, and the lowest rates found in China and other parts of Asia.[3,4] These differences are caused by genetic susceptibility, exposure to unknown external risk factor, or differences in health care and cancer registration, or even a combination of these factors.[4]

Cancer of the prostate is multifocal and it is commonly observed that the cancerous gland contains multiple independent lesions, suggesting the heterogeneity of the disease.[5] Determinants responsible for the pathologic growth of the prostate remain poorly understood, although steroidal androgens and peptide growth factors have been implicated.[6,7] As long as the cancer is confined to the prostate, it can be successfully controlled by surgery or radiation, but in metastatic disease, few options are available beyond androgen ablation,[8] the mainstay of treatment in the case of lymph node involvement or disseminated loci. Once tumor cells have become hormone refractory, the standard cytotoxic agents are marginally effective in slowing disease progression, although they do provide some degree of palliative relief. Current chemotherapeutic regimens, typically two or more agents, afford response rates in the range of only 20–30%.[9,10]

We have endeavored to address the need for improved antitumor therapy by means of a novel approach, and a recent report from our laboratory details the development of serine amide phosphates (SAPs), derivatives of lysophosphatidic acid (LPA) as effective cytotoxic agents against human prostate cancer cell lines.[11] We showed that SAP derivatives represent a class of anticancer agents for the treatment of prostate cancer and several of these were potent inhibitors of prostate tumor cell proliferation at low micromolar concentrations.[11] Despite their high cytotoxicity, the same compounds were not selective against non-tumor CHO cells and LPA receptor negative RH7777 cells. We also hypothesize that the phosphate group in SAPs is susceptible to hydrolysis, as the phosphate moiety is readily hydrolyzed by the action of lipid phosphate

Keywords: Prostate cancer; Lysophosphatidic acid; Phosphate mimics; Thiazolidinones; Antiproliferative effect.
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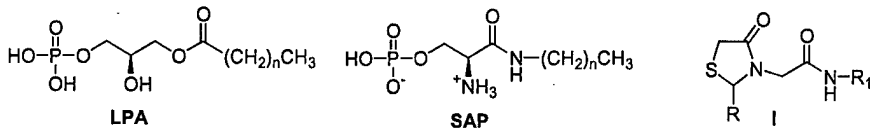
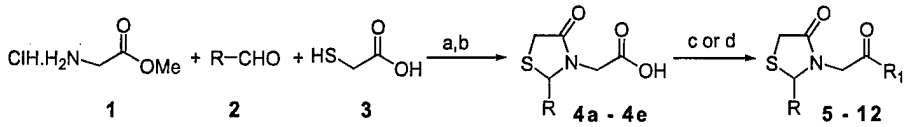


Figure 1.

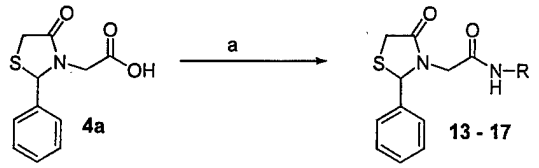


Scheme 1. Reagents: (a) toluene, Dean–Stark; (b) NaOH, MeOH; (c) $CH_3(CH_2)_nNH_2$, EDC, HOBt, CH_2Cl_2; (d) $(COCl)_2$, benzene, NH_3, MeOH.

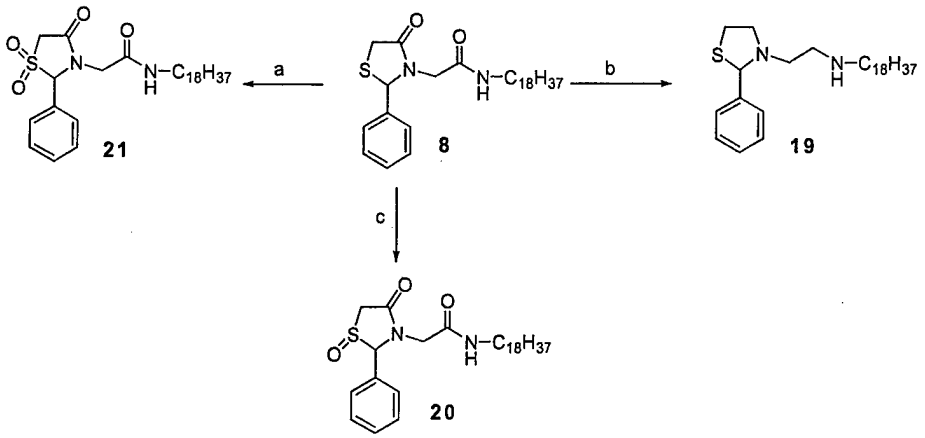
phosphatases.[12–14] The biomimetic replacement of phosphate group by a hydrolytically more stable pharmacophore would be expected to prolong biological activity by altering pharmacokinetics and metabolism.

Andres et al. have postulated that 4-thiazolidinones may be recognized as phosphate mimics, and using this scaffold they synthesized and evaluated some 4-thiazolidinones for their ability to inhibit the bacterial enzyme MurB.[15] Based on this hypothesis, we decided to explore the 4-thiazolidinone pharmacophore as a biomimetic replacement for the phosphate group. This strategic modification would be expected to enhance the physiochemical, pharmacokinetic, and antiproliferative properties and result in highly potent and selective anticancer agents for prostate cancer. To this end, we designed a new series of thiazolidinone derivatives as shown in Figure 1. In this paper we report the synthesis, structure–activity relationship, and antiproliferative activity of type **I** compounds (Fig. 1) in five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, and TSU).

The synthesis of thiazolidinone derivatives (**5–12**) utilized straightforward chemistry as shown in Scheme 1. Various 4-thiazolidinones were synthesized following a reported procedure[16] of condensing mercaptoacetic acid, glycine methyl ester, and aromatic aldehydes in a one-pot reaction, followed by basic hydrolysis of the ester. Thiazolidinone amides were obtained by the treatment with appropriate amines in the presence of EDC/HOBt under standard conditions. Compound **5** that has no side chain was synthesized from the corresponding acid as shown in Scheme 1. Thiazolidinone amides **13–17** were synthesized by a simple and direct method,[17] which involves reaction of the acid **4a** with different isocyanates in the presence of a catalytic amount of DMAP (Scheme 2). Exhaustive reduction of **8** using BH_3·THF under reflux conditions gave **19** (Scheme 3). Oxidation of **8** using H_2O_2 and with $KMnO_4$ afforded sulfoxide (**20**) and sulfone (**21**), respectively, as shown in Scheme 3. All compounds[18] were characterized by ^1H and ^{13}C NMR, mass spectroscopy and, in certain cases, elemental analysis.

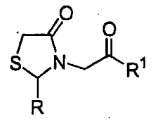


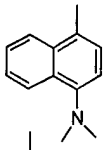
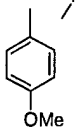
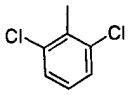
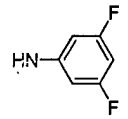
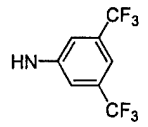
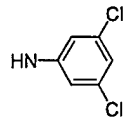
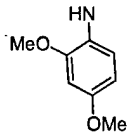
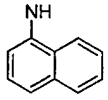
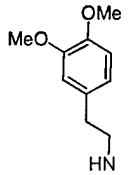
Scheme 2. Reagents: (a) R–NCO, DMAP, CH_2Cl_2.



Scheme 3. Reagents: (a) $KMnO_4$, AcOH; (b) BH_3·THF; (c) H_2O_2, AcOH.

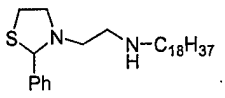
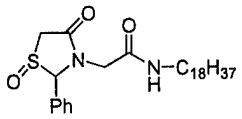
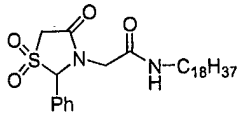
Table 1. Antiproliferative effects of compounds **4a–b** and **5–18**



Compd	R	R^1	IC_{50} (μM) RH 7777[a]	DU-145[b]	PC-3[b]	LNCaP[b]	PPC-1[b]	TSU[b]
4a	Ph	OH	ND	>50	>50	>50	>50	>50
4b	Biphenyl	OH	>100	>100	>100	>100	>100	>100
5	Ph	NH_2	>100	>100	>100	>100	>100	>100
6	Ph	$NHC_{10}H_{21}$	20.0	22.4	20.3	14.1	15.8	19.7
7	Ph	$NHC_{14}H_{29}$	16.4	19.6	13.5	14.1	10.1	13.4
8	Ph	$NHC_{18}H_{37}$	39.6	12.6	11.1	9.3	7.1	8.5
9	Biphenyl	$NHC_{18}H_{37}$	>50	>50	>50	>50	>50	>50
10		$NHC_{18}H_{37}$	>50	>50	>50	>50	>50	>50
11		$NHC_{18}H_{37}$	31.1	14.8	12.6	11.8	10.7	17.5
12		$NHC_{18}H_{37}$	>50	>50	>50	>50	>50	>50
13	Ph		70.9	69.0	74.1	24.1	46.2	53.2
14	Ph		25.4	16.2	18.1	14.5	13.1	16.1
15	Ph		34.9	24.0	28.6	13.2	20.5	17.2
16	Ph		>100	>100	>100	82.5	>100	60.8
17	Ph		>100	>100	>100	31.4	>100	69.9
18	Ph		>100	>100	>100	>100	>100	>100
	5-FU		ND	11.9	12.0	4.9	6.4	3.6

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

Table 2. Antiproliferative effects of compounds **19–21**

Compd	Structure	IC_{50} (μM)					
		RH 7777[a]	DU-145[b]	PC-3[b]	LNCaP[b]	PPC-1[b]	TSU[b]
19		>20	15.8	>20	>20	12.0	6.1
20		11.5	11.2	6.5	7.9	5.4	6.4
21		22.1	15.5	8.5	10.9	5.5	9.3
	5-FU	ND	11.9	12.0	4.9	6.4	3.6

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

The antiproliferative activity of all the synthesized compounds has been evaluated against five human prostate cancer cell lines and in RH7777 cells (negative control) using the sulforhodamine B (SRB) assay.[19] 5-Fluorouracil (5-FU) was used as reference drug. As shown in Table 1, 4-thiazolidinone carboxylic acids (**4a** and **4b**) were unable to inhibit the growth of any of the five prostate cancer cells below 50 μM. However, the corresponding amides (**6–8**) showed higher activities. It was observed that an increase in the alkyl chain length [**6** (C10), **7** (C14), and **8** (C18)] enhances the antiproliferative activity of these analogs in prostate cancer cells. Interestingly, the simple amide **5** without any long alkyl chain is not cytotoxic below 100 μM, which indicates that the absence of an alkyl side chain causes a considerable decrease in antiproliferative effect. On the other hand, replacement of the alkyl chain with various aryl side chains (**13–18**) reduced the biological activity. Among this series, **13** is moderately cytotoxic, where as analogs **16–18** displayed poor cytotoxicity in several prostate cancer cell lines. However, it is noteworthy to mention that thiazolidinone amides (**14** and **15**), with electron-withdrawing substituents on the aryl ring showed cytotoxicity in the range of 13–29 μM against all five prostate cancer cell lines.

Thiazolidinone derivatives (**9** and **10**) with bulky biphenyl or naphthalene groups demonstrated low cytotoxicity compared to **8** (Table 1). We synthesized compounds **11** and **12** to understand the effects of aromatic ring substitution in **8**. It was observed that electron-donating substituents maintained good activity while the *ortho* electron-withdrawing substituents substantially decrease the antiproliferative activity of these derivatives (Table 1). Compound **19**, which has no amide groups, showed significantly good potency in all five prostate cancer cell lines. Notably, **20** and **21** bearing sulfoxide or sulfone moiety displayed higher cytotoxic potency comparable to that of the reference drug 5-FU against both PC-3 and PPC-1 cell lines (Table 2).

In summary, starting with the SAPs, we identified a series of novel and cytotoxic 4-thiazolidinone amides based on a 4-thiazolidinone scaffold. Among this series, we synthesized and carried out detailed structure activity relationship studies of type **I** compounds (Fig. 1) and evaluated their antipropliferative activity against five prostate cancer cell lines and RH7777 cells (negative controls). The cytotoxicity study shows that the antiproliferative activity is sensitive to 2-aryl ring substitutions, the length of the alkyl side chain, and the removal or replacements of the lipophilic alkyl side chain. Sulfur oxidation is well tolerated as compounds **20** and **21** showed significant cytotoxicity compared to 5-FU. This study resulted in the discovery of potent cytotoxic 4-thiazolidinones **8**, **20**, and **21**, which inhibit the growth of all five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, and TSU) with 2–5-fold lower selectivity compared to RH7777 cell line. These 4-thiazolidinone derivatives are a significant improvement on the SAP moiety in that they are less cytotoxic but demonstrated improved selectivity in non-tumor cells. However, further investigations are required to increase the potency and selectivity.

Acknowledgements

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18. Compounds were obtained as mixtures of diastereomers and were used as such for the biological studies. Characteristic data for some compounds are given below. Compound **8**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.0 Hz, 3H), 1.26 (br s, 30H), 1.46 (m, 2H), 3.16–3.29 (m, 3H), 3.82 (d, J = 1.5 Hz, 2H), 4.20 (s, 0.5H), 4.25 (s, 0.5H), 5.83–5.85 (m, 2H), 7.27–7.41 (m, 5H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 13.55, 22.13, 26.30, 28.69, 28.80, 28.88, 28.99, 29.03, 29.10, 29.14, 31.37, 32.13, 39.08, 45.88, 63.67, 127.05, 128.58, 128.96, 137.61, 166.30, 171.61; MS (ESI) *m/z* 511 [M+Na]. Anal. Calcd for $C_{29}H_{48}N_2O_2S$: C, 71.26; H, 9.90; N, 5.73. Found: C, 71.18; H, 10.03; N, 5.79. Compound **11**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.0 Hz, 3H), 1.26 (br s, 30H), 1.33 (s, 2H), 3.16–3.19 (m, 1H), 3.2–3.29 (m, 2H), 3.80 (d, J = 0.9 Hz, 2H), 3.83 (s, 3H), 4.16 (s, 0.5H), 4.21 (s, 0.47H), 5.82 (s, 1H), 6.9 (dd, J = 1.8 Hz, 2H), 7.29 (dd, J = 1.5 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 13.53, 22.12, 26.31, 28.70, 28.74, 28.79, 28.89, 28.99, 29.03, 29.09, 29.13, 31.36, 32.23, 39.06, 45.74, 54.79, 63.44, 128.64, 129.11, 159.97, 166.41, 171.47; MS (ESI) *m/z* 541 [M+Na]. Anal. Calcd for $C_{30}H_{50}N_2O_3S$: C, 69.45; H, 9.71; N, 5.40. Found: C, 69.30; H, 9.86; N, 5.43. Compound **12**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.54 (d, J = 15.3 Hz, 1H), 3.87 (s, 2H), 4.25 (d, J = 15.3 Hz, 1H), 5.88 (s, 1H), 7.10 (t, J = 1.8 Hz, 1H), 7.36–7.43 (m, 7H), 8.29 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 32.35, 46.73, 64.40, 117.37, 123.85, 127.29, 128.74, 129.32, 134.59, 136.87, 138.61, 165.14, 172.60; MS (ESI) *m/z* 403 [M+Na]. Anal. Calcd for $C_{17}H_{14}Cl_2N_2O_2S$: C, 53.55; H, 3.70; N, 7.35. Found: C, 53.39; H, 3.47; N, 7.36. Compound **21**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.0 Hz, 3H), 1.26 (br s, 32H), 3.19–3.34 (m, 3H), 3.88–4.03 (dd, J = 16.5 Hz, 2H), 4.66 (s, 0.5H), 4.72 (s, 0.5H), 5.67 (br s, 1H), 5.95 (s, 1H), 7.38 (m, 2H), 7.50–7.53 (m, 3H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 13.54, 22.12, 26.26, 28.66, 28.79, 28.96, 29.02, 29.09, 29.14, 31.36, 39.30, 44.35, 49.85, 81.32, 125.77, 128.43, 128.91, 130.55, 163.23, 165.30; MS (ESI) *m/z* 519 [M−H]. Anal. Calcd for $C_{29}H_{48}N_2O_4S$: C, 66.88; H, 9.29; N, 5.38. Found: C, 66.68; H, 9.27; N, 5.41.
19. Thiazolidinone derivatives were dissolved in dimethyl sulfoxide (DMSO) and serially diluted in complete growth medium to desired final concentrations at DMSO concentrations of less than 0.5%. 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 and 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.

Efficient Microwave Enhanced Synthesis of 4 -Thiazolidinones

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Abstract: A microwave enhanced, rapid, 3-component one-pot condensation method has been developed for the synthesis of 4-thiazolidinones using environmentally benign solvent ethanol in open vessels at atmospheric pressure. Applying this methodology ten different 4-thiazolidinones were synthesized in good yields.

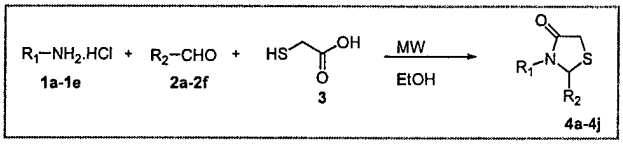
Key words: microwave, heterocycles, condensation, 4-thiazolidinones, synthesis

Experience has shown that compounds with biological activity are often derived from heterocyclic structures. Indeed, one of richest sources of diversity for the medicinal chemist is small heterocyclic rings, which in addition to often exhibiting biological activity, may serve as rigid scaffolds for further display of functionalities. Thiazolidinones are one such class of heterocycles, which attracted much attention as they have been reported to possess a wide range of biological activities including antifungal, antibacterial, antihistaminic, antimicrobial and anti-inflammatory activities[1].

As part of our endeavor to discover new anticancer agents we designed and synthesized highly cytotoxic thiazolidinone scaffold containing compounds for prostate cancer (undisclosed results). During this process we were seeking a rapid and efficient method for the synthesis of 4-thiazolidinones. Literature survey shows that many different protocols have been developed for the synthesis of 4-thiazolidinones. Most commonly employed methods[2] involve a one-pot three-component condensation of a primary amine, an aldehyde and mercaptoacetic acid with simultaneous azeotropic distillation of water formed in the reaction. Alternatively, a recent report[3] describes carbodiimide (DCC) mediated three-component reaction for the synthesis of 4-thiazolidinones. In either case the reaction is believed to proceed via imine formation followed by attack of sulfur on the imine carbon and final intramolecular cyclization with the elimination of water. However, the general applicability of the above-mentioned methods are limited, as the reactions require prolonged heating with continuous removal of water, and in some cases the reaction is performed in sealed vessels in the presence of a desiccant like anhydrous $ZnCl_2$[4] or sodium sulphate[5] or molecular sieves, and the use of stoichiometric amounts of DCC. In order to circumvent these difficulties and to speed up the synthesis we focused on developing an alternate method for the synthesis of 4-thiazolidinones.

Initially introduced in 1986[6] the chemical application of microwaves has now become an area of interest for the synthesis of a wide variety of compounds. The advantages of microwave-expedited chemical synthesis are cleaner reactions, shorter reaction times and the ease of manipulation. Parekh[7] has described microwave-mediated synthesis of 4-thiazolidinones. However, this method[7] involves separate preparation of an hydrazone from the corresponding aldehyde and hydrazine, which was then mixed with thioglycolic acid and exposed to high power microwave irradiation.

In this communication we report microwave enhanced three-component one-pot condensation of primary amine, an aldehyde and mercaptoacetic acid for the synthesis of a diverse set of 4-thiazolidinones (Scheme 1).



Scheme 1

A range of primary amines and aldehydes were condensed with mercaptoacetic acid in the presence of Hunig's base and molecular sieves under microwave irradiation (Figure 1). To optimize the method, initially we examined the condensation in toluene and observed that desired product was formed in low yield. Furthermore, all our attempts to improve the yield at elevated temperature, microwave power and longer reaction times were met with unsuccessful results. This may be probably due to poor microwave absorbing nature of toluene. In microwave mediated organic synthesis one of the most important characteristics of a solvent is its polarity. The more polar a solvent, the greater its ability to couple with the microwave energy, the faster the temperature of the reaction mixture increases that leads to faster reaction rates. To increase the efficiency we decided to perform the condensation in a more polar and high microwave absorbing solvent like ethanol. However, most of the reported methods[2] involve use of either high boiling hydrocarbons like toluene or benzene, or aprotic solvent tetrahydrofuran for this type of condensations. To check the feasibility of condensation in a protic solvent, a pilot experiment was carried out using glycine, benzaldehyde and mercaptoacetic acid (Table 1, entry 1) in ethanol and observed that the reaction proceeded uneventfully forming the desired product in good yield. Interestingly, no product was formed when the reaction was carried out in ethanol in the absence of microwaves.

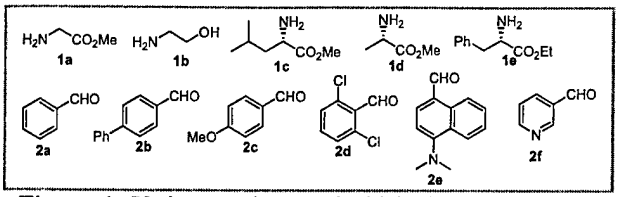


Figure 1. Various amines and aldehydes used for the condensation

Encouraged by this result and to understand the general applicability of this protocol, we have synthesized a variety of 4-thiazolidinones. For these purpose five different amines, five different aldehydes were selected and condensed with mercaptoacetic acid (Table 1). With a chiral center in the amine component (Table 1, entries 8-10) as one might expect, formation of diastereomeric products was observed. The diastereomeric ratio was determined by NMR and LC-MS analysis {**4h** (1:1.8), **4i** (1:1), **4j** (1:1.7)}. It was observed that the ratios of reactants at 1:2:3 for amine, aldehyde and mercaptoacetic acid, respectively gave best yields. This is in agreement with the earlier observation by Holmes et al.[2] Accordingly, the optimized procedure[8] involves microwave irradiation (power: 100 W) of a mixture of amine, aldehyde and mercapto acetic acid (1:2:3) in presence of 1.25 equiv of Hunig's base in ethanol at 120°C for 30 min at atmospheric pressure and after standard workup gave the desired 4-thiazolidinones in good to high yields (Table 1).

In conclusion, we have developed a convenient three-component one-pot microwave rate enhanced efficient method for the synthesis of 4-thiazolidinones. It is noteworthy to mention that all reactions were carried out at atmospheric pressure in open vessels using environmentally benign solvent ethanol. The simplicity of this short procedure and generally satisfactory yields render this method particularly attractive for the rapid synthesis of 4-thiazolidinones.

Table 1 Isolated Yields of 4-Thiazolidinones

Entry	Amine.HCl	Aldehyde	Mercapto acid	4-Thiazolidinone	%Yield
1	1a	2a	3	4a	80
2	1a	2b	3	4b	83
3	1a	2c	3	4c	90
4	1a	2d	3	4d	65
5	1a	2e	3	4e	80
6	1a	2f	3	4f	91
7	1b	2a	3	4g	76
8	1c	2a	3	4h	55
9	1d	2a	3	4i	68
10	1e	2a	3	4j	63

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(8) Typical procedure for the synthesis of 4-thiazolidinones: A mixture of Glycine methyl ester hydrochloride (**1a**, 0.5 g, 4 mmol), aldehyde (**2e**, 1.66 g, 8.3 mmol), mercaptoacetic acid (0.83 mL, 12 mmol), diisopropylethylamine (0.85 mL, 4.83 mmol), and molecular sieves (4A°, 0.1 g) in ethanol (10 mL) was irradiated with microwaves (power: 100W) at 120° C for 30 min, following which the sample was cooled using compressed air. The reaction mixture was diluted with $CHCl_3$ (75 mL), sequentially washed with satd. $NaHCO_3$, water, brine, dried (Na_2SO_4) and solvent was removed in vacuo to get crude product that was purified by column chromatography (silica gel, hexanes-ethyl acetate) to afford **4e** (1 g, 80%). ^1H NMR (300 MHz, $CDCl_3$) δ 2.85-2.86 (m, 1H), 2.92 (s, 6H), 3.5-3.63 (s, 3H), 3.83 (bs, 2H), 4.64 (d, J = 17.1 Hz, 1H), 6.67 (bs, 0.6 H), 7.07 (bs, 0.7H), 7.34 (d, J = 9 Hz, 1H), 7.55 (d, J = 3.3 Hz, 2H), 7.90 (bs, 1H), 8.31 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 31.94, 43.75, 44.53, 51.76, 58.47, 76.72, 112.97, 121.63, 122.69, 124.83, 124.94, 126.18, 131.33, 168.09; MS (ESI) *m/z* 345 [M+H].

Table of Contents Graphic

Discovery of 2-Arylthiazolidine-4-carboxylic acid amides as a New Class of Cytotoxic Agents for Prostate Cancer

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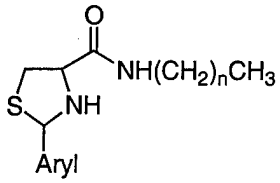

Title

Discovery of 2-Arylthiazolidine-4-carboxylic acid amides as a New Class of Cytotoxic Agents for Prostate Cancer[¶]

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[¶] Part of this work was presented at 227th ACS National Meeting, Anaheim, CA, March 28-April 1, 2004 and at 95th AACR Annual Meeting, Orlando, FL, March 27-March 31, 2004.

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Abstract

We showed that serine amide phosphates (SAPs), derivatives of lysophosphatidic acid (LPA) represent a class of cytotoxic phospholipids that are effective and potent in killing prostate cancer cells (Gududuru et al., *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4919-4923). We examined the cytotoxicity of a large number of SAPs in different prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, and TSU-Pr1), and in a negative control cell line (RH7777) that lacks LPA receptor. Although many of these compounds showed significant cytotoxicity, they were non-selective. To improve the selectivity and antiproliferative activity, we designed a new series of 4-thiazolidinone amides (Gududuru et al., *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5289-5293), in which the 4-thiazolidinone moiety was introduced as a phosphate mimic. However, these 4-thiazolidinone derivatives demonstrated less cytotoxicity in prostate cancer cells despite improved selectivity over RH7777 cells. To further optimize the thiazolidinone pharmacophore in terms of cytotoxicity and selectivity, we made closely related structural modifications on 4-thiazolidinone amides, which led us to the discovery of a new class of 2-arylthiazolidine-4-carboxylic acid amides. These compounds were potent cytotoxic agents with IC_{50} values in the low micromolar concentration range and demonstrated enhanced selectivity in receptor-negative cells compared to SAPs and 4-thiazolidinone amides.

Introduction

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. G-protein coupled receptors (GPCRs) are a family of membrane-bound proteins that are involved in the proliferation and survival of prostate cancer cells initiated by binding of lysophospholipids (LPLs).[1-4] The importance of G protein-dependent pathways in the regulation of growth and metastasis *in vivo* is corroborated by the observation that the growth of androgen-independent prostate cancer cells in mice is attenuated by treatment with pertussis toxin, an inhibitor of Gi/o proteins.[5] Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are lipid mediators generated via the regulated breakdown of membrane phospholipids that are known to stimulate GPCR-signaling.

LPLs bind to GPCRs encoded by the *Edg* gene family, collectively referred to as LPL receptors, to exert diverse biological effects. Lysophosphatidic acid (LPA) stimulates phospholipase D activity and PC-3 prostate cell proliferation.[6] Further, prior studies have shown that LPA is mitogenic in prostate cancer cells and that PC-3 and DU-145 cells express LPA_1, LPA_2, and LPA_3 receptors.[7] Advanced prostate cancers express LPL receptors and depend on phosphatidylinositol 3-kinase (PI3K) signaling for growth and progression to androgen independence.[2] Thus, these pathways are widely viewed as one of the most promising new approaches to cancer therapy,[8] and provide an especially novel approach to the treatment of advanced, androgen-refractory prostate cancer. Despite the promise of this approach, there are no clinically available therapies that selectively exploit or inhibit LPA or PI3K signaling.

We have been exploring a novel series of molecules that inhibit the growth of human prostate cancer cells. In a previous contribution from our laboratory,[9] by replacing the glycerol backbone in LPA with serine amide, effective cytotoxic agents were obtained, which showed potent cytotoxicity, comparable to that of reference drug 5-fluorouracil in five prostate cancer cell lines. However, the most potent compounds in that series of derivatives were non-selective and potently killed both prostate cancer and control cell lines. To improve the selectivity, enhance the pharmacokinetic, and antiproliferative properties, we designed a new series of 2-aryl-4-oxo-thiazolidin amides with general structure **III** (Figure 1), utilizing 4-thiazolidinone pharmacophore as a biomimetic replacement for the phosphate group.[10] This strategic modification showed that the 2-arylthiazolidinone moiety is indeed quite beneficial for obtaining a new set of antiproliferative compounds with improved selectivity, but resulted in decreased potency compared to SAPs.[9] To further optimize the structural characteristics of these compounds to selectively elicit antiproliferative activity, we made closely related, minor modifications to 2-aryl-4-oxo-thiazolidin amides as shown in Figure 1. Our current work highlights synthesis, structure activity relationship (SAR) studies, and biological evaluation of 2-arylthiazolidine-4-carboxylic acid amides (ATCAAs) for prostate cancer.

Chemistry

Compounds described in this study were prepared following straightforward chemistry. Reaction of L-cysteine with various aldehydes under reported conditions[11] gave corresponding acids, which were isolated as diastereomeric mixtures. These mixtures were used directly for the formation of corresponding amides by reacting with appropriate alkyl amines using EDC/HOBt as shown in Scheme 1. All compounds thus prepared were characterized as diastereomeric mixtures (Table 1). N-Acyl and N-sulfonyl derivatives (**28** and **29**) were synthesized from **5** by standard procedures (Scheme 2). The synthesis of thiazole derivative **34** was accomplished starting from cysteine as shown in Scheme 3.

The structures of the synthesized compounds and the yields of the syntheses are presented in Table 1.

Results and Discussion

The ability of 2-aryl-thiazolidine derivatives (ATCAAs) to inhibit the growth of five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, and TSU-Pr1) was assessed using the sulforhodamine B (SRB) assay.[9] We also included a control cell line (RH7777) that does not express LPL receptors,[12] to understand whether the antiproliferative activity of these derivatives was mediated through inhibition of LPL receptors. We first examined LPL receptor expression in these cell lines by RT-PCR to validate their use as *in vitro* models (Table 2). LPA_1 was the predominant LPL receptor expressed in these cell lines. However, LNCaP cells did not express this receptor subtype. LPA_2 was also expressed in all prostate cancer cell lines examined. Interestingly, ovarian cancer cells also demonstrated overexpression of LPA_2 compared to normal ovarian epithelial cells.[8] PC-3 and LNCaP cells, but not DU-145 cells, expressed LPA_3, consistent with published data.[7] None of the LPL receptors was expressed in RH7777 cells.

The diastereomeric mixtures of the target compounds **3-29** were used as such to evaluate their *in vitro* inhibitory activity against prostate cancer cell lines, and the results are summarized in Tables 3 and 4. 5-Fluorouracil was used as a reference drug for comparison. To deduce sound structure-activity relationships, IC_{50}s should on principle be determined on pure isomers. One drawback of testing mixtures of stereoisomers, unavoidable in our case, was that we could not assess how each stereoisomer affected the biological activity. On the other hand, the IC_{50} values calculated can be used as a screening method to select promising selective cytotoxic agents and to identify the diastereomeric mixture with the most potent ability to inhibit the growth of prostate cancer cells. Many of these thiazolidine analogs were very effective in killing prostate

cancer cell lines with IC_{50} values as low as 480 nM (Table 3). Examination of the cytotoxic effects of **3-5** showed that as the chain length increased from C_7 to C_{18}, the potency also increased. However, a further increase in the alkyl chain length by one carbon unit (**6**) caused a significant loss of activity. Interestingly, the C_{14} derivative (**4**) demonstrated higher potency than **5**, but was 8-fold less selective against the RH7777 cell line. Thus, an alkyl chain with C_{18} unit was optimal for maintaining the potency and selectivity observed in this series of compounds. N-Acyl and N-sulfonyl derivatives (**28** and **29**) were significantly less cytotoxic than parent compound **5**. Replacement of the phenyl ring with an alkyl or cyclohexyl group reduced the potency (**7** and **8**) relative to the thiazolidine derivative (**5**). Introduction of a methylene spacer separating the phenyl ring and the thiazolidine ring furnished a compound **9**, which was less active than the parent compound **5**.

To understand the effect of unsaturation on potency and selectivity, and to overcome the problems associated with stereoisomers, we replaced the central thiazolidine core in **5** with a thiazole ring. However, thiazole derivative (**34**) did not show any activity below 20 μM in both prostate and RH7777 cells, which suggests that thiazolidine ring with two chiral centers plays an important role in providing potency and selectivity. Replacements of the phenyl ring with a heterocycle, such as an indole, pyridine or furan ring was investigated by synthesizing analogs **10-12**. The furanyl derivative **12** showed equivalent cytotoxicity as **5**, but was 3-fold less selective against RH7777 cells.

The cytotoxicity data of compounds **13-27** provides a summary of a broad survey of phenyl ring substituted analogs. Examination of the IC_{50} values of these analogs demonstrates a greater tolerance for diverse substituents in the phenyl ring. In general, the most potent analogues possessed electron-donating substituents, as exemplified by

comparison of **13** and **16-18**, relative to **5**. Compound **18** is one of the most active compounds with an IC_{50} of 0.55 μM was 38-fold more selective in PPC-1 cells compared to RH7777 cells. On the other hand, thiazolidine analogs (**19-25**), with electron-withdrawing substituents demonstrated less cytotoxicity. Comparison of the potencies of **26** and **27**, suggest that substitution of the phenyl ring with a bulky group reduces the activity.

From the LPL receptor mRNA expression studies (Table 2), it was evident that these cell lines serve as an excellent model system to explore the effects of LPL receptors in prostate cancer cell growth. Given the structural similarity of SAPs to ceramide (and the known ability of ceramide to induce apoptosis), we next determined whether the antiproliferative effects of thiazolidine analogs were mediated via apoptotic events. We examined the ability of our analogs to induce apoptosis in LNCaP, PC-3, and RH7777 cells using a quantitative sandwich ELISA[13] that measures DNA-histone complex released during apoptosis. The enrichment factor calculated as ratio of OD405 in treated and un-treated cells provides a quantitative assessment of the degree of apoptosis induced. Initially, we used only two compounds (**4** and **5**) for this study. Apoptotic activity of analog **4** was selective in prostate cancer cells despite nonselective cytotoxicity in RH7777 negative control cells (Table 5). Analog **5** induced apoptosis in PC-3 and LNCaP cells, but to a lesser extent in PC-3 cells perhaps due to lower potency in this cell line. This data suggests that thiazolidine analogs may act as potent inducers of apoptosis and selectively kill a variety of prostate cancer cell lines.

Conclusions

2-Aryl-thiazolidine-4-carboxylic acid amides (ATCAAs) were obtained by the modification of previously reported 4-thiazolidinones. We synthesized a number of ATCAAs

and evaluated for their inhibitory activity towards the growth of human prostate cancer cell lines. Introduction of ring activating groups on the phenyl ring resulted in increasing potencies for prostate cancer cell lines leading to discovery of several new anticancer agents represented by analogues **16**, **17**, and **18** with low/sub micromolar cytoxicity and high selectivity. From this study, compound **18** emerged as one of the most potent and selective cytotoxic agents with an IC_{50} of 0.55 μM and 38-fold selectivity in PPC-1 cells. Further, the ability of these analogs to induce apoptosis in LNCaP and PC-3 cells provides an important clue to understand their mechanism of action, and suggests that they may have therapeutic utility in the treatment of prostate or ovarian cancer. All compounds discussed in this report have been prepared and tested as diastereomeric mixtures. Future efforts shall aim at synthesis and evaluation of pure individual stereoisomers of the most promising thiazolidines discussed above.

Experimental Section

All reagents and solvents used were reagent grade or were purified by standard methods before use. Moisture-sensitive reactions were carried under an argon atmosphere. Progress of the reactions was followed by thin-layer chromatography (TLC) analysis. Flash column chromatography was carried out using silica gel (200-425 mesh) supplied by Fisher. Melting points were measured in open capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. All compounds were characterized by NMR and MS (ESI). ^{1}H NMR spectra were recorded on a Varian 300 instrument. Chemical shifts are reported as δ values relative to Me_4Si as internal standard. Mass spectra were obtained in the electrospray (ES) mode using Esquire-LC (Bruker) spectrometer. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA).

General Procedure for the Preparation of 3-27. A mixture of appropriate carboxylic acid (**2a-2v**, 0.3-0.5 g), EDC (1.25 eq) and HOBt (1 eq) in CH_2Cl_2 (25-50 mL) was stirred for 10 min. To this solution, appropriate alkyl amine (1 equiv) was added and stirring continued at room temperature for 6-8 h. Reaction mixture was diluted with CH_2Cl_2 (100-150 mL) and sequentially washed with water, sat. $NaHCO_3$, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to yield a crude solid, which was purified by column chromatography. The purified compounds (**3-6**, **12**, **15-18** & **27**) were converted to corresponding hydrochlorides using 2M HCl/Et_2O.

(2*RS*, 4*R*)-2-Phenylthiazolidine-4-carboxylic acid heptylamide Hydrochloride (3.HCl). ^{1}H NMR (DMSO-d_6) δ 8.72 (s, 1H), 7.65 (m, 2H), 7.43 (m, 3H), 5.89 (s, 0.6H), 5.84 (s, 0.4H), 4.66 (t, J = 6.3 Hz, 0.6H), 4.46 (t, J = 6.9 Hz, 0.4H), 3.55-3.71 (m, 1H), 3.24-3.34 (m, 1H), 3.13 (d, J = 5.7 Hz, 2H), 1.44 (m, 2H), 1.25 (s, 8H), 0.83 (t, J = 6.9 Hz, 3H); MS (ESI) *m/z* 307.10 (M+1).

(2*RS*, 4*R*)-2-Phenylthiazolidine-4-carboxylic acid tetradecylamide Hydrochloride (4.HCl). ^{1}H NMR (DMSO-d_6) δ 8.69 (m, 1H), 7.64-7.71 (m, 2H), 7.45 (m, 3H), 5.89 (s, 0.6H), 5.84 (s, 0.4H), 4.67 (t, J = 6.6 Hz, 0.6H), 4.47 (t, J = 7.2 Hz, 0.4H), 3.55-3.71 (m, 1H), 3.25-3.35 (m, 1H), 3.10-3.16 (m, 2H), 1.44 (m, 2H), 1.23 (s, 22H), 0.85 (t, J = 6.3 Hz, 3H); MS (ESI) *m/z* 427.30 (M+Na).

(2*RS*, 4*R*)-2-Phenylthiazolidine-4-carboxylic acid octadecylamide Hydrochloride (5.HCl). ^{1}H NMR (DMSO-d_6) δ 8.59 (d, J = 5.1 Hz, 1H), 7.63 (d, J = 3.9 Hz, 2H), 7.42-7.47 (m, 3H), 5.86 (s, 0.6H), 5.81(s, 0.4H), 4.60 (t, J = 6.3 Hz, 0.6H), 4.39 (t, J = 6.9 Hz, 0.4H), 3.52-3.66 (m, 1H), 3.24-3.30 (m, 1H), 3.10-3.16 (m, 2H), 1.42 (m, 2H), 1.23 (s, 30H), 0.85 (t, J = 6.3 Hz, 3H); MS (ESI) *m/z* 461.50 (M+1).

(2*RS*, 4*R*)-2-Phenylthiazolidine-4-carboxylic acid nonadecylamide Hydrochloride (6.HCl). ^{1}H NMR (DMSO-d_6) δ 8.51 (s, 1H), 7.62 (m, 2H), 7.41-7.46 (m, 3H), 5.83 (s, 0.6H), 5.78 (s, 0.4H), 4.53 (m, 0.6H), 4.32 (m, 0.4H), 3.48-3.61 (m, 1H), 3.24-3.29 (m, 1H), 3.11-3.15 (m, 2H), 1.43 (m, 2H), 1.23 (s, 32H), 0.85 (t, J = 6.3 Hz, 3H); MS (ESI) *m/z* 497.40 (M+Na).

(2*RS*, 4*R*)-2-Dodecylthiazolidine-4-carboxylic acid octadecylamide (7). ^{1}H NMR ($CDCl_3$) δ 7.18 (m, 1H), 4.20-4.27 (m, 1H), 3.79 (m, 0.3H), 3.54-3.59 (m, 0.7H), 3.08-3.34 (m, 4H), 1.65-1.78 (m, 2H), 1.43-1.51 (m, 4H), 1.27 (brs, 48H), 0.89 (t, J = 6 Hz, 6H); MS (ESI) *m/z* 553.60 (M+1).

(2*RS*, 4*R*)-2-Cyclohexylthiazolidine-4-carboxylic acid octadecylamide (8). ^{1}H NMR ($CDCl_3$) δ 7.17 (m, 1H), 4.10-4.20 (m, 1H), 3.76 (m, 0.3H), 3.54 (dd, J = 11.1, 3.6 Hz, 0.7H), 2.97-3.34 (m, 4H), 2.02 (m, 1H), 1.68-1.78 (m, 4H), 1.48-1.54 (m, 2H), 1.27 (brs, 36H), 0.87 (t, J = 6.9 Hz, 3H); MS (ESI) *m/z* 467.60 (M+1).

(2*RS*, 4*R*)-2-Benzylthiazolidine-4-carboxylic acid octadecylamide (9). ^{1}H NMR ($CDCl_3$) δ 7.28-7.33 (m, 5H), 7.03 (s, 0.7H), 6.48 (s, 0.3H), 4.55 (brs, 0.5H), 4.18 (brs, 0.5H), 3.82 (brs, 0.3H), 3.54 (dd, *J* = 11.1, 3.6 Hz, 0.7H), 2.99-3.31 (m, 6H), 1.46-1.51 (m, 2H), 1.27 (brs, 30H), 0.89 (t, *J* = 6.3 Hz, 3H); MS (ESI) *m/z* 475.50 (M+1).

(2*RS*, 4*R*)-2-(1*H*-Indol-3yl)-thiazolidine-4-carboxylic acid octadecylamide (10). ^{1}H NMR ($CDCl_3$) δ 7.86 (m, 0.6H), 7.77 (m, 0.4H), 7.41-7.48 (m, 4H), 7.29-7.34 (m, 1H), 6.0 (s, 0.3H), 5.69 (s, 0.7H), 4.37-4.41 (m, 0.5H), 3.76 (dd, *J* = 11.1, 4.2 Hz, 0.5H), 3.23-3.52 (m, 3H), 2.79-3.04 (m, 1H), 1.43 (m, 2H), 1.27 (s, 30H), 0.89 (t, *J* = 6.6 Hz, 3H); MS (ESI) *m/z* 500.60 (M+1).

(2*RS*, 4*R*)-2-Pyridin-3-yl-thiazolidine-4-carboxylic acid octadecylamide (11). ^{1}H NMR ($CDCl_3$) δ 8.74 (d, *J* = 2.1 Hz, 1H), 8.60 (d, *J* = 4.8 Hz, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 7.31-7.36 (m, 1H), 7.08 (m, 1H), 5.44 (s, 0.5H), 5.40 (s, 0.5H), 4.28-4.35 (m, 1H), 3.72 (dd, *J* = 11.1, 4.2 Hz, 1H), 3.27-3.45 (m, 3H), 2.57 (m, 1H), 1.53-1.57 (m, 2H), 1.26 (s, 30H), 0.89 (t, *J* = 6.6 Hz, 3H); MS (ESI) *m/z* 462.40 (M+1).

(2*RS*, 4*R*)-2-Furan-3-yl-thiazolidine-4-carboxylic acid Hydrochloride (12.HCl). ^{1}H NMR (DMSO-d_6) δ 8.59 (d, *J* = 15.6 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.72 (s, 1H), 5.86 (s, 0.7H), 5.78 (s, 0.3H), 4.37-4.56 (m, 1H), 3.50-3.63 (m, 1H), 3.11-3.23 (m, 3H), 1.43 (m, 2H), 1.23 (s, 30H), 0.85 (t, *J* = 6.6 Hz, 3H); MS (ESI) *m/z* 451.60 (M+1).

(2*RS*, 4*R*)-2-(4-Dimethylamino-phenyl)-thiazolidine-4-carboxylic acid octadecylamide (13). ^{1}H NMR ($CDCl_3$) δ 7.34-7.41 (m, 2H), 6.70-6.74 (m, 2H), 5.57 (s, 0.3H), 5.28 (s, 0.7H), 4.34 (m, 0.7H), 3.90 (m, 0.3H), 3.69 (dd, *J* = 11.1, 4.2 Hz, 1H), 3.41-3.47 (m, 1H), 3.20-3.33 (m, 2H), 2.97 (d, *J* = 3.6 Hz, 6H), 1.48-1.55 (m, 2H), 1.27 (s, 30H), 0.89 (t, *J* = 6.3 Hz, 3H); MS (ESI) *m/z* 504.60 (M+1).

(2*RS*, 4*R*)-2-(3-Hydroxy-phenyl)-thiazolidine-4-carboxylic acid octadecylamide (14). ^{1}H NMR (DMSO-d_6) δ 8.59 (s, 1H), 7.22 (t, J = 6.6 Hz, 1H), 7.02 (d, J = 6.3 Hz, 2H), 6.82 (d, J = 7.5 Hz, 1H), 5.77 (s, 0.7H), 5.71 (s, 0.3H), 4.545 (m, 0.7H), 4.37 (m, 0.3H), 3.49-3.59 (m, 1H), 3.13-3.27 (m, 3H), 1.43 (brs, 2H), 1.23 (s, 30H), 0.85 (t, J = 6.3 Hz, 3H); MS (ESI) *m/z* 477.60 (M+1).

(2*RS*, 4*R*)-2-(4-Methoxy-phenyl)-thiazolidine-4-carboxylic acid octadecylamide Hydrochloride (15.HCl). ^{1}H NMR (DMSO-d_6) δ 8.61 (m, 1H), 7.57 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 9 Hz, 2H), 5.83 (s, 0.7H), 5.78 (s, 0.3H), 4.61 (t, J = 6.3 Hz, 0.7H), 4.40 (m, 0.3H), 3.77 (s, 3H), 3.51-3.70 (m, 1H), 3.22-3.31 (m, 1H), 3.11 (m, 2H), 1.43 (m, 2H), 1.23 (s, 30H), 0.84 (t, J = 6.6 Hz, 3H); MS (ESI) *m/z* 491.60 (M+1).

(2*RS*, 4*R*)-2-(3,4-Dimethoxy-phenyl)-thiazolidine-4-carboxylic acid octadecylamide Hydrochloride (16.HCl). ^{1}H NMR (DMSO-d_6) δ 8.58 (m, 1H), 7.33 (d, J = 4.2 Hz, 1H), 7.14 (t, J = 7.5 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 5.81 (s, 0.8H), 5.77 (s, 0.2H), 4.62 (m, 0.7H), 4.40 (m, 0.3H), 3.78 (d, J = 7.8 Hz, 6H), 3.52-3.68 (m, 1H), 3.23-3.29 (m, 1H), 3.12-3.13 (m, 2H), 1.43 (m, 2H), 1.23 (s, 30H), 0.85 (t, J = 6.6 Hz, 3H); MS (ESI) *m/z* 521.60 (M+1).

(2*RS*, 4*R*)-2-(3,4,5-Trimethoxy-phenyl)-thiazolidine-4-carboxylic acid octadecylamide Hydrochloride (17.HCl). ^{1}H NMR (DMSO-d_6) δ 8.59 (m, 1H), 7.01 (d, J = 5.7 Hz, 2H), 5.80 (s, 0.8H), 5.76 (s, 0.2H), 4.63 (m, 0.7H), 4.37 (m, 0.3H), 3.80 (d, J = 5.7 Hz, 6H), 3.66 (s, 3H), 3.23-3.28 (m, 1H), 3.12-3.13 (m, 2H), 1.43 (m, 2H), 1.23 (s, 30H), 0.85 (t, J = 6 Hz, 3H); MS (ESI) *m/z* 551.60 (M+1).

(2*RS*, 4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid octadecylamide Hydrochloride (18.HCl). ^{1}H NMR (DMSO-d_6) δ 10.18 (s, 1H), 8.61 (m, 1H), 7.54-7.64 (m, 4H), 5.82 (s, 0.7H), 5.77 (s, 0.3H), 4.60 (m, 0.8H), 4.42 (m, 0.2H), 3.56-3.64 (m, 1H), 3.12-

3.26 (m, 3H), 2.05 (s, 3H), 1.43 (m, 2H), 1.23 (s, 30H), 0.84 (t, J = 6 Hz, 3H); MS (ESI) *m/z* 518.70 (M+1).

(2*RS*, 4*R*)-2-(4-Fluoro-phenyl)-thiazolidine-4-carboxylic acid octadecylamide (19). ^{1}H NMR ($CDCl_3$) δ 7.46-7.54 (m, 2H), 7.13-7.20 (m, 1H), 7.01-7.08 (m, 2H), 5.60 (s, 0.3H), 5.34 (s, 0.7H), 4.76 (m, 0.3H), 4.34 (m, 0.7H), 3.69 (dd, J = 11.1, 6.9 Hz, 1H), 3.21-3.52 (m, 3H), 1.49 (m, 2H), 1.26 (s, 30H), 0.89 (t, J = 6.3 Hz, 3H); MS (ESI) *m/z* 479.60 (M+1).

(2*RS*, 4*R*)-2-(4-Bromo-phenyl)-thiazolidine-4-carboxylic acid octadecylamide (20). ^{1}H NMR ($CDCl_3$) δ 7.48-7.62 (m, 2H), 7.36-7.42 (m, 2H), 7.14 (m, 0.7H), 6.40 (m, 0.3), 5.57 (d, J = 10.2 Hz, 0.3H), 5.33 (d, J = 11.1 Hz, 0.7H), 4.32 (m, 0.7H), 3.94 (m, 0.3H), 3.70 (dd, J = 11.1, 4.2 Hz, 1H), 3.20-3.44 (m, 3H), 1.49 (m, 2H), 1.27 (s, 30H), 0.89 (t, J = 6.3 Hz, 3H); MS (ESI) *m/z* 539.70 (M^+).

(2*RS*, 4*R*)-2-(4-Nitro-phenyl)-thiazolidine-4-carboxylic acid octadecylamide (21). ^{1}H NMR ($CDCl_3$) δ 8.24 (d, J = 8.7 Hz, 2H), 7.67 (d, J = 8.7 Hz, 2H), 6.92 (m, 1H), 5.54 (s, 0.5H), 5.50(s, 0.5H), 4.24-4.31 (m, 1H), 3.67 (dd, J = 10.8, 4.8 Hz, 1H), 3.27-3.44 (m, 3H), 1.55 (m, 2H), 1.26 (s, 30H), 0.89 (t, J = 6.3 Hz, 3H); MS (ESI) *m/z* 506.60 (M+1).

(2*RS*, 4*R*)-2-(4-Cyano-phenyl)-thiazolidine-4-carboxylic acid octadecylamide (22). ^{1}H NMR ($CDCl_3$) δ 7.60-7.70 (m, 4H), 6.94 (m, 0.6H), 6.37 (m, 0.4), 5.64 (s, 0.4H), 5.46 (s, 0.6H), 4.27 (m, 0.6H), 3.96 (m, 0.4H), 3.65-3.70 (m, 1H), 3.20-3.45 (m, 3H), 1.54 (m, 2H), 1.26 (s, 30H), 0.89 (t, J = 6.3 Hz, 3H); MS (ESI) *m/z* 508.50 (M+Na).

(2*RS*, 4*R*)-2-(3,5-Difluoro-phenyl)-thiazolidine-4-carboxylic acid octadecylamide (23). ^{1}H NMR ($CDCl_3$) δ 7.04-7.08 (m, 2H), 6.97 (m, 1H), 6.79 (m, 1H), 5.40 (s, 0.5H), 5.36 (s, 0.5H), 4.23-4.30 (m, 1H), 3.66 (dd, J = 11.1, 4.5 Hz, 1H), 3.26-3.42 (m, 3H), 1.33 (m, 2H), 1.26 (s, 30H), 0.89 (t, J = 6.3 Hz, 3H); MS (ESI) *m/z* 497.50 (M+1).

(2*RS*, 4*R*)-2-(2,6-Dichloro-phenyl)-thiazolidine-4-carboxylic acid octadecylamide (24). ^{1}H NMR ($CDCl_3$) δ 7.34-7.38 (m, 2H), 7.15-7.28 (m, 2H), 6.29 (s, 0.5H), 6.25 (s, 0.5H), 4.25 (t, *J* = 5.7 Hz, 1H), 3.94 (dd, *J* = 10.5, 1.8 Hz, 1H), 3.26-3.52 (m, 3H), 1.52 (m, 2H), 1.26 (s, 30H), 0.89 (t, *J* = 6 Hz, 3H); MS (ESI) *m/z* 529.70 (M^+).

(2*RS*, 4*R*)-2-(3-Bromo-4-fluoro-phenyl)-thiazolidine-4-carboxylic acid octadecylamide (25). ^{1}H NMR ($CDCl_3$) δ 7.71 (m, 1H), 7.42 (m, 1H), 7.06-7.16 (m, 2H), 5.56 (d, *J* = 9.3 Hz, 0.2H), 5.34 (d, *J* = 10.2 Hz, 0.8H), 4.29 (d, *J* = 4.5 Hz, 0.8H), 3.94 (m, 0.2H), 3.69 (dd, *J* = 11.1, 4.2 Hz, 1H), 3.21-3.41 (m, 3H), 1.52 (m, 2H), 1.26 (s, 30H), 0.89 (t, *J* = 6.3 Hz, 3H); MS (ESI) *m/z* 558.70 (M+1).

(2*RS*, 4*R*)-2-*p*-Tolyl-thiazolidine-4-carboxylic acid octadecylamide (26). ^{1}H NMR ($CDCl_3$) δ 7.34-7.43 (m, 2H), 7.14-7.21 (m, 3H), 5.59 (s, 0.2H), 5.32 (s, 0.8H), 4.76 (m, 0.2H), 4.35 (m, 0.8H), 3.70 (dd, *J* = 11.1, 3.9 Hz, 1H), 3.21-3.43 (m, 3H), 2.36 (d, *J* = 2.7 Hz, 3H), 1.51 (m, 2H), 1.27 (s, 30H), 0.89 (t, *J* = 6.3 Hz, 3H); MS (ESI) *m/z* 475.60 (M+1).

(2*RS*, 4*R*)-2-Biphenyl-4-yl-thiazolidine-4-carboxylic acid octadecylamide Hydrochloride (27.HCl). ^{1}H NMR (DMSO-d_6) δ 8.59 (m, 1H), 7.66-7.73 (m, 5H), 7.37-7.51 (m, 4H), 5.92 (s, 0.7H), 5.87 (s, 0.3H), 4.62 (m, 0.7H), 4.41 (m, 0.3H), 3.53-3.64 (m, 1H), 3.26-3.32 (m, 1H), 3.13-3.17 (m, 2H), 1.44 (m, 2H), 1.22 (s, 30H), 0.84 (t, *J* = 6.3 Hz, 3H); MS (ESI) *m/z* 537.70 (M+1).

(2*RS*, 4*R*)-3-Acetyl-2-phenylthiazolidine-4-carboxylic acid octadecylamide (28). ^{1}H NMR ($CDCl_3$) δ 7.31-7.41 (m, 5H), 6.01 (s, 1H), 5.12 (s, 1H), 3.73 (m, 1H), 3.40 (m, 1H), 3.31 (m, 1H), 3.11-3.17 (m, 1H), 2.00 (s, 3H), 1.27-1.33 (m, 32H), 0.89 (t, *J* = 6.3 Hz, 3H); MS (ESI) *m/z* 502.60 (M^+).

(2*RS*, 4*R*)-3-Methanesulfonyl-2-phenylthiazolidine-4-carboxylic acid octadecylamide (29). ^{1}H NMR ($CDCl_3$) δ 7.65-7.68 (m, 2H), 7.32-7.36 (m, 3H), 6.20 (s, 1H), 4.63 (dd, J = 9, 6 Hz, 1H), 3.67 (dd, J = 12, 6 Hz, 1H), 3.47 (dd, J = 12.3, 8.1 Hz, 1H), 3.04-3.13 (m, 2H), 3.02 (s, 3H), 1.27 (m, 32H), 0.89 (t, J = 6.3 Hz, 3H); MS (ESI) *m/z* 538.70 (M^+).

2-Phenylthiazolidine-4-carboxylic acid methyl ester (31). To a solution of DL-cysteine (3g, 24.76 mmol) in MeOH (50 mL) at 0°C, $SOCl_2$ (2.76 mL, 37.14 mmol) was slowly added and warmed to room temperature then refluxed for 3 h. The reaction mixture was concentrated in vacuo to yield a residue. This residue was taken in to aqueous EtOH (1:1, 30 mL), $NaHCO_3$ (2.28 g, 27.23 mmol) was added, after 10 min benzaldehyde (2.5 mL, 24.76 mmol) was added and stirring continued for 3 h. $CHCl_3$ (200 mL) was added to the reaction mixture and washed with water, brine, dried (Na_2SO_4) and solvent was removed in vacuo. The crude product was purified by column chromatography to afford **31** (4.7 g, 85%). ^{1}H NMR ($CDCl_3$) δ 7.51-7.62 (m, 2H), 7.32-7.42 (m, 3H), 5.84 (s, 0.4H), 5.58 (s, 0.6H), 4.24 (t, J = 6.3 Hz, 0.4H), 4.01 (t, J = 7.5 Hz, 0.6H), 3.83 (s, 3H), 3.39-3.55 (m, 1H), 3.10-3.26 (m, 1H); MS (ESI) *m/z* 224 (M+1).

2-Phenylthiazole-4-carboxylic acid methyl ester (32). Compound **32** was synthesized following a reported procedure.[14] Yield (0.33 g, 68%). ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H), 8.0-8.04 (m, 2H), 7.45-7.50 (m, 3H), 4.0 (s, 3H); MS (ESI) *m/z* 220 (M+1).

2-Phenylthiazole-4-carboxylic acid octadecylamide (34). To a solution of **32** (0.5 g, 2.28 mmol) in MeOH (10 mL) at 0°C, 1N NaOH (5 mL) was added and stirred for 2 h. To the reaction mixture EtOAc (30 mL) was added and acidified with 1N HCl. Extracted with EtOAc (3 X 50 mL), combined extracts were washed with water, brine, dried (Na_2SO_4) and solvent was removed under vacuo to give crude acid **33**, which was converted to **34** (0.30 g, 68%), following the general procedure used as in the case of synthesis of **3-27**. ^{1}H NMR ($CDCl_3$) δ

8.10 (s, 1H), 7.96-7.93 (m, 2H), 7.46-7.50 (m, 3H), 3.49 (dd, J = 13.5, 6.9 Hz, 2H), 1.69 (m, 2H), 1.27 (m, 30H), 0.89 (t, J = 6.3 Hz, 3H); MS (ESI) m/z 457.60 (M+1).

Cell Culture. DU-145, PC-3, and LNCaP human prostate cancer cells, and RH7777 rat hepatoma cells were obtained from American Type Culture Collection (Manassas, VA). Dr. Mitchell Steiner at University of Tennessee Health Science Center kindly provided PPC-1 and TSU-Pr1 cells. Prostate cancer cells and RH7777 cells were maintained in RPMI 1640 medium and DMEM (Mediatech, Inc., Herndon, VA), respectively, supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY) in 5% CO_2 / 95% air humidified atmosphere at 37 °C.

RT-PCR Analysis of LPA Receptor Expression. Total RNA was extracted using TRIzol® reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instruction. 0.5 μg (LPA_1) or 1 μg (LPA_2 and LPA_3) of total RNA was used to perform RT-PCR using SuperScript™ One-Step RT-PCR with Platinum® *Taq* (Invitrogen Corp., Carlsbad, CA) with 0.2 μM of primers. The following primer pairs were used: LPA_1 forward 5′-GCTCCACACACGGATGAGCAACC-3′, LPA_1 reverse 5′-GTGGTCATTGCTGTGAACTCCAGC-3′; LPA_2 forward 5′-CTGCTCAGCCGCTCCTATTTG-3′, LPA_2 reverse 5′-AGGAGCACCCACAAGTCATCAG-3′; LPA_3 forward 5′-CCATAGCAACCTGACCAAAAAGAG-3′, LPA_3 reverse 5′-TCCTTGTAGGAGTAGATGATGGGG-3′; β-actin forward 5′-GCTCGTCGTCGACAACGGCTC-3′, β-actin reverse 5′-CAAACATGATCTGGGTCATCTTCTC-3′. PCR conditions were as follows: After 2 min denaturation step at 94 °C, samples were subjected to 34 to 40 cycles at 94 °C for 30

sec, 60 °C (LPA_1) or 58 °C (LPA_2 and LPA_3) for 30 sec, and 72 °C for 1 min, followed by an additional elongation step at 72 °C for 7 min. Primers were selected to span at least one intron of the genomic sequence to detect genomic DNA contamination. The PCR products were separated on 1.5% agarose gels, stained with ethidium bromide, and the band intensity was quantified using Quantity One Software (Bio-Rad Laboratories, Inc., Hercules, CA). Expression levels of each receptor subtype in different cell lines were expressed as ratios compared to β-actin mRNA level.

Cytotoxicity Assay. For in vitro cytotoxicity screening, 1000 to 5000 cells were plated into each well of 96-well plates depending on growth rate, and exposed to different concentrations of a test compound for 96 h in three to five replicates. All the compounds were dissolved in dimethyl sulfoxide at 5 to 20 mM, and diluted to desired concentrations in complete culture medium. Cell numbers at the end of the drug treatment were measured by the SRB assay. Briefly, the cells were fixed with 10% of trichloroacetic acid, stained with 0.4% SRB, and the absorbances at 540 nm was measured using a plate reader (DYNEX Technologies, Chantilly, VA). Percentages of cell survival versus drug concentrations were plotted and the IC_{50} (concentration that inhibited cell growth by 50% of untreated control) values were obtained by nonlinear regression analysis using WinNonlin (Pharsight Corporation, Mountain View, CA). 5-fluorouracil was used as a positive control to compare potencies of the new compounds.

Apoptosis. A sandwich ELISA (Roche, Mannheim, Germany) utilizing monoclonal antibodies specific for DNA and histones was used to quantify degree of apoptosis induced by the analogs after 72 h exposure. This assay measures DNA-histone complexes (mono- and oligonucleosomes) released into cytoplasm from the nucleus during apoptosis.

RH7777 cells were employed because of nonspecific cytotoxicity of compound **4** in receptor-negative cells as well as receptor-positive prostate cancer cells.

Acknowledgements

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Supporting Information Available: General procedure for the preparation of compounds **2a-2v** along with spectral data is available free of charge via the Internet at http://pubs.acs.org.

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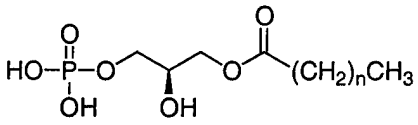
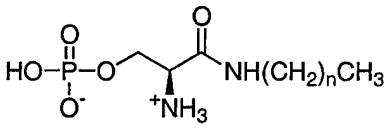
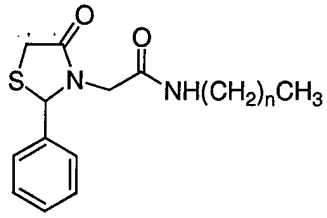
Figure 1



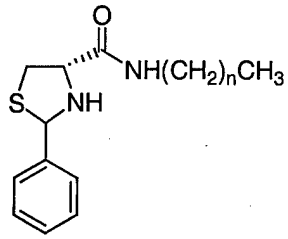
LPA (**I**)



Serine Amide Phosphate (**II**)

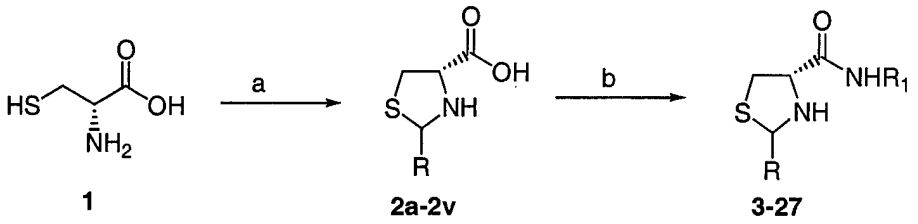


2-Aryl-4-oxo-thiazolidin amide (**III**)



2-Arylthiazolidine-4-carboxylic acid amide (**IV**)

Scheme 1[a]

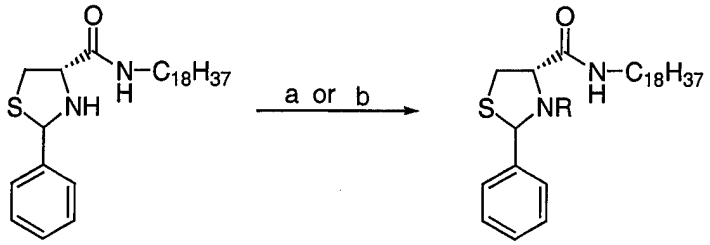


1 **2a-2v** **3-27**

Compd	R
2a	phenyl
2b	n-dodecyl
2c	cycohexyl
2d	benzyl
2e	3-indolyl
2f	3-pyridinyl
2g	3-furanyl
2h	4-dimethyl amino phenyl
2i	3-hydroxyphenyl
2j	4-methoxyphenyl
2k	3,4-dimethoxyphenyl
2l	3,4,5-trimethoxyphenyl
2m	4-acetylamino phenyl
2n	4-fluorophenyl
2o	4-bromophenyl
2p	4-nitrophenyl
2q	4-cyanophenyl
2r	3,5-difluorophenyl
2s	2,6-dichlorophenyl
2t	3-bromo-4-fluorophenyl
2u	4-methylphenyl
2v	biphenyl

[a]Reagents and conditions: (a) RCHO, EtOH; (b) $CH_3(CH_2)_nNH_2$, EDC, HOBt, CH_2Cl_2

Scheme 2[a]

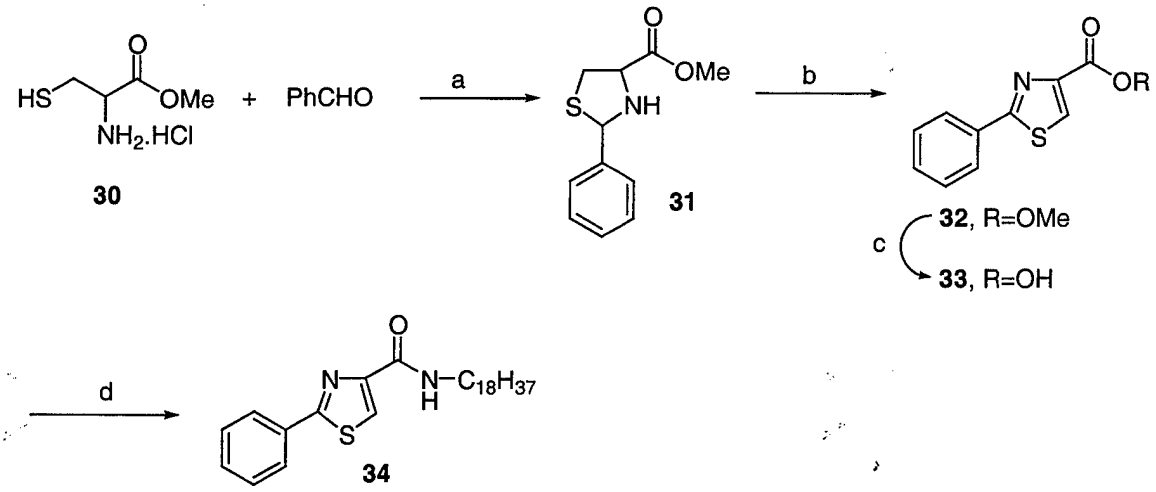


5

28, R=$COCH_3$
29, R=SO_2CH_3

[a]Reagents and conditions: (a) Ac_2O, Pyridine; (b) CH_3SO_2Cl, Pyridine

Scheme 3[a]



30

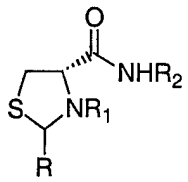
31

32, R=OMe
33, R=OH

34

[a]Reagents and conditions: (a) $NaHCO_3$, EtOH, H_2O; (b) NBS, CCl_4; (c) NaOH, MeOH; (d) $C_{18}H_{37}NH_2$, EDC, HOBt, CH_2Cl_2

Table 1. Structures and Physical Data of Synthesized Compounds



compd	R	R_1	R_2	mp (°C)	yield (%)	formula	anal.
3.HCl	phenyl	H	C_7H_{15}	ND	80	$C_{17}H_{27}ClN_2OS$	C, H, N
4.HCl	phenyl	H	$C_{14}H_{29}$	95	83	$C_{24}H_{41}ClN_2OS$	C, H, N
5.HCl	phenyl	H	$C_{18}H_{37}$	93	70	$C_{28}H_{49}ClN_2OS$	C, H, N
6.HCl	phenyl	H	$C_{19}H_{39}$	85	78	$C_{29}H_{51}ClN_2OS$	C, H, N
7	n-dodecyl	H	$C_{18}H_{37}$	86	69	$C_{34}H_{68}N_2OS$	C, H, N
8	cycohexyl	H	$C_{18}H_{37}$	60	75	$C_{28}H_{54}N_2OS$	C, H, N
9	benzyl	H	$C_{18}H_{37}$	80	81	$C_{29}H_{50}N_2OS$	C, H, N
10	3-indolyl	H	$C_{18}H_{37}$	125	65	$C_{30}H_{49}N_3OS$	C, H, N
11	3-pyridinyl	H	$C_{18}H_{37}$	94	63	$C_{27}H_{47}N_3OS$	C, H, N
12.HCl	3-furanyl	H	$C_{18}H_{37}$	99	60	$C_{26}H_{47}ClN_2O_2S$	C, H, N
13	4-dimethyl amino phenyl	H	$C_{18}H_{37}$	75	75	$C_{30}H_{53}N_3OS$	C, H, N
14	3-hydroxyphenyl	H	$C_{18}H_{37}$	50	69	$C_{28}H_{48}N_2O_2S$	C, H, N
15.HCl	4-methoxyphenyl	H	$C_{18}H_{37}$	95	70	$C_{29}H_{51}ClN_2O_2S$	C, H, N
16.HCl	3,4-dimethoxyphenyl	H	$C_{18}H_{37}$	103	83	$C_{30}H_{53}ClN_2O_3S$	C, H, N
17.HCl	3,4,5-trimethoxyphenyl	H	$C_{18}H_{37}$	115	70	$C_{31}H_{55}ClN_2O_4S$	C, H, N
18.HCl	4-acetylamino phenyl	H	$C_{18}H_{37}$	170	63	$C_{30}H_{52}ClN_3O_2S$	C, H, N
19	4-fluorophenyl	H	$C_{18}H_{37}$	65	73	$C_{28}H_{47}FN_2OS$	C, H, N
20	4-bromophenyl	H	$C_{18}H_{37}$	81	77	$C_{28}H_{47}BrN_2OS$	C, H, N
21	4-nitrophenyl	H	$C_{18}H_{37}$	115	60	$C_{28}H_{47}N_3O_3S$	C, H, N
22	4-cyanophenyl	H	$C_{18}H_{37}$	90	70	$C_{29}H_{47}N_3OS$	C, H, N
23	3,5-difluorophenyl	H	$C_{18}H_{37}$	113	70	$C_{28}H_{46}F_2N_2OS$	C, H, N
24	2,6-dichlorophenyl	H	$C_{18}H_{37}$	49	80	$C_{28}H_{46}Cl_2N_2OS$	C, H, N

25	3-bromo-4-fluorophenyl	H	$C_{18}H_{37}$	100	78	$C_{28}H_{46}BrFN_2OS$	C, H, N
26	4-methylphenyl	H	$C_{18}H_{37}$	120	73	$C_{29}H_{50}N_2OS$	C, H, N
27.HCl	biphenyl	H	$C_{18}H_{37}$	130	70	$C_{34}H_{53}ClN_2OS$	C, H, N
28	phenyl	$COCH_3$	$C_{18}H_{37}$	90	95	$C_{30}H_{50}N_2O_2S$	C, H, N
29	phenyl	SO_2CH_3	$C_{18}H_{37}$	55	90	$C_{29}H_{50}N_2O_3S_2$	C, H, N

Table 2. LPL Receptor mRNA Expression

LPL Receptor	Old name	Expression level relative to β-actin					
		RH7777	DU145	PC-3	LNCaP	PPC-1	TSU-Pr1
LPA_1	EDG-2	UD[a]	2.16	2.53	UD	2.29	2.13
LPA_2	EDG-4	UD	0.33	0.43	0.32	0.41	0.19
LPA_3	EDG-7	UD	0.07	0.27	0.28	0.15	UD
Sum LPA_{1-3}		0	2.56	3.23	0.60	2.85	2.32

[a]UD: under detection limit

Table 3. Antiproliferative Effects of Compounds **3-18**

Compd	IC_{50} (μM)					
	RH7777[a]	DU-145[b]	PC-3[b]	LNCaP[b]	PPC-1[b]	TSU-Pr1[b]
3.HCl	52.2	44.9	38.5	12.4	34.7	28.0
4.HCl	3.4	2.4	3.0	1.4	1.3	2.0
5.HCl	25.6	5.4	7.8	2.1	2.0	5.0
6.HCl	No activity	> 20	No activity	13.6	16.8	> 20
7	~20	8.9	15.0	11.9	13.0	10.7
8	> 20	> 20	> 20	12.8	9.3	> 20
9	> 20	15.3	16.4	4.4	4.0	11.2
10	> 20	8.9	11.5	2.1	1.3	4.4
11	10.5	7.5	9.2	3.6	2.9	7.8
12.HCl	10.4	6.6	8.1	1.7	1.1	4.2
13	> 20	5.3	6.0	1.6	1.1	3.0
14	31.0	5.7	6.7	1.7	1.2	4.0
15.HCl	>20	8.7	~20	2.1	1.5	ND
16.HCl	10.3	4.5	5.2	0.85	0.58	2.4
17.HCl	11.4	3.9	4.0	0.82	0.48	2.4
18.HCl	21.1	3.1	5.6	1.3	0.55	0.94
5-FU	ND	11.9	12.0	4.9	6.4	3.6

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

Table 4. Antiproliferative Effects of Compounds **19-29** and **34**

Compd	IC_{50} (μM)					
	RH7777[a]	DU-145[b]	PC-3[b]	LNCaP[b]	PPC-1[b]	TSU-Pr1[b]
19	17.4	5.7	6.8	1.9	2.1	5.4
20	> 20	13.8	17.3	5.1	3.7	18.3
21	~ 20	15.3	~ 20	8.4	15.3	15.9
22	>20	>20	>20	5.9	5.0	>20
23	>20	>20	>20	11.2	10.6	>20
24	> 20	> 20	> 20	13.1	17.1	~ 20
25	~ 20	11.3	13.5	3.0	4.7	14.0
26	> 20	10.5	12.8	1.9	1.9	8.0
27.HCl	>20	>20	>20	>20	>20	>20
28	>20	~20	~20	16.1	12.6	>20
29	>20	>20	>20	>20	>20	>20
34	>20	>20	>20	>20	>20	>20
5-FU	ND	11.9	12.0	4.9	6.4	3.6

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

Table 5. Thiazolidine amides- Induced Apoptosis

Compd for 72 h		PC-3	LNCaP	RH7777
4	2 μM	1.8	14.1	2.6
	5 μM	18.7	75.4	3.2
	10 μM	54.0	80.7	2.5
5	2 μM	1.4	4.5	ND[a]
	5 μM	2.3	45.2	
	10 μM	3.4	37.1	
	20 μM	12.7	26.1	

[a]ND: not determined

Supporting Information

Discovery of 2-Arylthiazolidine-4-carboxylic acid amides as a New Class of Cytotoxic Agents for Prostate Cancer

Veeresa Gududuru, Eunju Hurh, James T. Dalton, and Duane D. Miller *

Contents: General procedure for the preparation of compounds **2a-2v** and their ^{1}H NMR (300 MHz) and MS (ESI) characterization data.

General Procedure for the Preparation of 2a-2v. A mixture of L-cysteine (**1**, 0.5g, 4.12 mmol) and appropriate aldehyde (4.12 mmol) in ethanol (15 mL) was stirred at room temperature for 5 h, the solid separated was collected, washed with diethyl ether and dried to afford **2a-2v**.

(2*RS*, 4*R*)-2-Phenylthiazolidine-4-carboxylic acid (2a). Obtained as colorless crystals (0.82 g, 95%). ^{1}H NMR (DMSO-d_6) δ 7.24-7.53 (m, 5H), 5.67 (s, 0.6H), 5.50 (s, 0.4H), 4.22 (dd, J = 6.9, 4.5 Hz, 0.6H), 3.90 (dd, J = 8.7, 7.2 Hz, 0.4H), 3.27-3.40 (m, 1H), 3.04-3.16 (m, 1H); MS (ESI) *m/z* 208 (M-1).

(2*RS*, 4*R*)-2-Dodecylthiazolidine-4-carboxylic acid (2b). Obtained as colorless powder (0.87 g, 70%). ^{1}H NMR (DMSO-d_6) δ 4.53 (t, J = 6.9 Hz, 0.4H), 4.38 (t, J = 6.9 Hz, 0.6H), 4.03 (t, J = 6.9 Hz, 0.5H), 3.64-3.69 (m, 0.5H), 3.41-3.45 (m, 0.5H), 3.05-3.21 (m, 0.5H), 2.76-2.92 (m, 1H), 1.66-1.71 (m, 2H), 1.23 (brs, 20H), 0.85 (t, J = 6 Hz, 3H); MS (ESI) *m/z* 300 (M- 1).

(2*RS*, 4*R*)-2-Cyclohexylthiazolidine-4-carboxylic acid (2c). Colorless solid (0.88 g, 100%). ^{1}H NMR (DMSO-d_6) δ 4.34 (d, J = 8.4 Hz, 0.6H), 4.23 (d, J = 8.1 Hz, 0.4H), 4.0 (t, J = 5.7 Hz, 0.5H), 3.64-3.70 (m, 0.5H), 3.12-3.17 (m, 0.5H), 2.97-3.0 (m, 0.5H), 2.82-2.89 (m, 0.5H), 2.65

(t, J = 9 Hz, 0.5H), 1.95 (m, 1H), 1.55-1.66 (m, 4H), 0.89-1.21 (m, 6H); MS (ESI) *m/z* 214 (M- 1).

(2*RS*, 4*R*)-2-Benzylthiazolidine-4-carboxylic acid (2d). Colorless solid (0.73 g, 80%). ^{1}H NMR (DMSO-d_6) δ 7.17-7.29 (m, 5H), 4.81 (t, J = 6.9 Hz, 0.5H), 4.64 (t, J = 7.2 Hz, 0.5H), 4.12 (t, J = 6.6 Hz, 0.5H), 3.67-3.73 (m, 0.5H), 2.93-3.28 (m, 4H); MS (ESI) *m/z* 222 (M- 1).

(2*RS*, 4*R*)-2-(1*H*-Indol-3yl)-thiazolidine-4-carboxylic acid (2e). Yield (0.71 g, 70%). ^{1}H NMR (DMSO-d_6) δ 11.10 (m, 1H), 6.97-7.65 (m, 5H), 5.94 (s, 0.5H), 5.80 (s, 0.5H), 4.27-4.31 (m, 0.5H), 3.89 (t, J = 7.8 Hz, 0.5H), 2.78-3.47 (m, 2H); MS (ESI) *m/z* 247, (M- 1).

(2*RS*, 4*R*)-2-Pyridin-3-yl-thiazolidine-4-carboxylic acid (2f). Yield (0.32 g, 63%). ^{1}H NMR (DMSO-d_6) δ 8.68 (d, J = 2.1 Hz, 0.5H), 8.61 (d, J = 2.1 Hz, 0.5H), 8.52 (dd, J = 4.8, 1.8 Hz, 0.5H), 8.46 (dd, J = 4.8, 1.5 Hz, 0.5H), 7.95-7.99 (m, 0.5H), 7.81-7.85 (m, 0.5H), 7.34-7.42 (m, 1H), 5.74 (s, 0.5H), 5.55 (s, 0.5H), 4.18 (m, 0.5H), 3.91 (m, 0.5H), 3.29-3.40 (m, 1H), 3.0-3.15 (m, 0.5H); MS (ESI) *m/z* 209 (M- 1).

(2*RS*, 4*R*)-2-Furan-3-yl-thiazolidine-4-carboxylic acid (2g). Yield (0.64 g, 78%). ^{1}H NMR (DMSO-d_6) δ 7.77 (s, 1H), 7.55 (m, 1H), 6.57 (s, 0.5H), 6.51 (s, 0.5H), 5.59 (s, 0.5H), 5.43 (s, 0.5H), 4.16 (t, J = 6.6 Hz, 0.5H), 3.83 (J = 8.4 Hz, 0.5H), 3.23-3.35 (m, 1H), 2.96-3.15 (m, 1H); MS (ESI) *m/z* 198 (M- 1).

(2*RS*, 4*R*)-2-(4-Dimethylamino-phenyl)-thiazolidine-4-carboxylic acid (2h). Yield (1.04 g, 100%). ^{1}H NMR (DMSO-d_6) δ 7.24-7.31 (m, 2H), 6.68 (t, J = 8.4 Hz, 2H), 5.54 (s, 0.5H), 5.39 (s, 0.5H), 4.23-4.27 (m, 0.5H), 3.81 (t, J = 8.4 Hz, 0.5H), 3.26-3.46 (m, 1H), 2.93-3.15 (m, 1H), 2.88 (d, J = 8.4 Hz, 6H); MS (ESI) *m/z* 251 (M- 1).

(2*RS*, 4*R*)-2-(3-Hydroxy-phenyl)-thiazolidine-4-carboxylic acid (2i). Yield (0.65 g, 70%). ^{1}H NMR (DMSO-d_6) δ 7.08-7.18 (m, 1H), 6.82-6.91 (m, 2H), 6.63-6.74 (m, 1H), 5.59 (s,

0.5H), 5.41 (s, 0.5H), 4.16-4.20 (m, 0.5H), 3.83-3.89 (m, 0.5H), 3.24-3.39 (m, 1H), 3.0-3.17 (m, 1H); MS (ESI) *m/z* 224 (M- 1).

(2*RS*, 4*R*)-2-(4-Methoxy-phenyl)-thiazolidine-4-carboxylic acid (2j). Yield (0.98 g, 100%). ^{1}H NMR (DMSO-d_6) δ 7.42 (d, J = 8.7 Hz, 1H), 7.34 (d, J = 8.7 Hz, 1H), 6.93 (d, J = 3 Hz, 1H), 6.87 (d, J = 3 Hz, 1H), 5.60 (s, 0.5H), 5.44 (s, 0.5H), 4.21-4.24 (m, 0.5H), 3.81-3.87 (m, 0.5H), 3.75 (d, J = 4.2 Hz, 3H), 2.81-3.46 (m, 2H); MS (ESI) *m/z* 238 (M- 1).

(2*RS*, 4*R*)-2-(3,4-Dimethoxy-phenyl)-thiazolidine-4-carboxylic acid (2k). Yield (0.99 g, 90%). ^{1}H NMR (DMSO-d_6) δ 6.86-7.15 (m, 3H), 5.57 (s, 0.5H), 5.43 (s, 0.5H), 4.26-4.30 (m, 0.5H), 3.73-3.76 (m, 6H), 3.26-3.37 (m, 1H), 3.03-3.18 (m, 1H); MS (ESI) *m/z* 268 (M- 1).

(2*RS*, 4*R*)-2-(3,4,5-Trimethoxy-phenyl)-thiazolidine-4-carboxylic acid (2l). Yield (0.98 g, 80%). ^{1}H NMR (DMSO-d_6) δ 6.86 (s, 1H), 6.77 (s, 1H), 5.58 (s, 0.5H), 5.43 (s, 0.5H) 4.27-4.31 (m, 0.5H), 3.83-3.88 (m, 0.5H), 3.76 (d, J = 3.3 Hz, 6H) 3.64 (d, J = 3.3 Hz, 3H), 3.26-3.36 (m, 1H), 3.04-3.18 (m, 1H); MS (ESI) *m/z* 298 (M- 1).

(2*RS*, 4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid (2m). Yield (1.09 g, 100%). ^{1}H NMR (DMSO-d_6) δ 9.97 (d, J = 15 Hz, 1H), 7.50-7.57 (m, 2H), 7.42 (d, J = 8.7 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H), 5.60 (s, 0.5H), 5.44 (s, 0.5H), 4.23-4.27 (m, 0.5H), 3.81 (m, 0.5H), 3.25-3.46 (m, 1H), 3.05-3.16 (m, 1H), 2.03 (d, J = 2.4 Hz, 3H); MS (ESI) *m/z* 265 (M-1).

(2*RS*, 4*R*)-2-(4-Fluoro-phenyl)-thiazolidine-4-carboxylic acid (2n). Yield (0.45 g, 81%). ^{1}H NMR (DMSO-d_6) δ 7.55-7.60 (m, 1H), 7.46-7.50 (m, 1H), 7.11-7.23 (m, 2H), 5.67 (s, 0.5H), 5.50 (s, 0.5H), 4.19-4.23 (m, 0.5H), 3.86-3.91 (m, 0.5H), 3.27-3.39 (m, 1H), 3.05-3.16 (m, 1H); MS (ESI) *m/z* 226 (M-1).

(2*RS*, 4*R*)-2-(4-Bromo-phenyl)-thiazolidine-4-carboxylic acid (2o). Yield (0.66 g, 93%). ^{1}H NMR (DMSO-d_6) δ 7.47-7.58 (m, 3H), 7.38 (d, J = 8.4 Hz, 1H), 5.67 (s, 0.5H), 5.49 (s, 0.5H), 4.14-4.18 (m, 0.6H), 3.86-3.91 (m, 0.4H), 3.26-3.38 (m, 1H), 3.04-3.13 (m, 1H); MS (ESI) *m/z* 287 (M- 1).

(2*RS*, 4*R*)-2-(4-Nitro-phenyl)-thiazolidine-4-carboxylic acid (2p). Yield (0.40 g, 65%). ^{1}H NMR (DMSO-d_6) δ 8.20 (dd, J = 12, 8.7 Hz, 2H), 7.80 (d, J = 8.7 Hz, 1H), 7.67 (d, J = 8.7 Hz, 1H), 5.94 (s, 0.7H), 5.88 (s, 0.3H), 3.91-4.11 (m, 1H), 3.03-3.12 (m, 1H), 2.77-2.93 (m, 1H); MS (ESI) *m/z* 253 (M- 1).

(2*RS*, 4*R*)-2-(4-Cyano-phenyl)-thiazolidine-4-carboxylic acid (2q). Yield (0.40 g, 70%). ^{1}H NMR (DMSO-d_6) δ 7.68-7.85 (m, 2H), 7.60 (d, J = 8.1 Hz, 2H), 5.80 (s, 0.7H), 5.59 (s, 0.3H), 4.11 (t, J = 6 Hz, 0.8H), 3.90-3.96 (m, 0.2H), 3.27-3.38 (m, 1H), 3.04-3.11 (m, 1H); MS (ESI) *m/z* 233 (M- 1).

(2*RS*, 4*R*)-2-(3,5-Difluoro-phenyl)-thiazolidine-4-carboxylic acid (2r). Yield (0.46 g, 77%). ^{1}H NMR (DMSO-d_6) δ 7.30 (d, J = 6.6 Hz, 2H), 7.08-7.17 (m, 1H), 5.53 (s, 1H), 3.89-3.94 (m, 1H), 3.27-3.35 (m, 1H), 3.12 (t, J = 18 Hz, 1H); MS (ESI) *m/z* 244 (M- 1).

(2*RS*, 4*R*)-2-(2,6-Dichloro-phenyl)-thiazolidine-4-carboxylic acid (2s). Yield (0.47 g, 69%). ^{1}H NMR (DMSO-d_6) δ 7.55 (d, J = 0.9 Hz, 1H), 7.53 (s, 1H), 7.33-7.47 (m, 1H), 6.25 (s, 1H), 3.89 (dd, J = 9.9, 6.6 Hz, 1H), 3.47 (dd, J = 9.9, 6.6 Hz, 1H), 2.99 (t, J = 10.2 Hz, 1H); MS (ESI) *m/z* 277 (M- 1).

(2*RS*, 4*R*)-2-(3-Bromo-4-fluoro-phenyl)-thiazolidine-4-carboxylic acid (2t). Yield (0.5 g, 66%). ^{1}H NMR (DMSO-d_6) δ 7.93 (dd, J = 6.9, 2.1 Hz, 0.3H), 7.77 (dd, J = 6.6, 1.8 Hz, 0.7H), 7.45-7.59 (m, 1H), 7.35 (m, 1H), 5.68 (s, 0.7H), 5.50 (s, 0.3H), 4.16 (q, J = 6.6 Hz, 0.7H), 3.88 (q, J = 9 Hz, 0.3H), 3.24-3.36 (m, 1H), 3.06-3.13 (m, 1H); MS (ESI) *m/z* 305 (M- 1).

(2*RS*, 4*R*)-2-*p*-Tolyl-thiazolidine-4-carboxylic acid (2u). Yield (0.49 g, 90%). ^{1}H NMR (DMSO-d_6) δ 7.39 (d, J = 8.1 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.12-7.19 (m, 2H), 5.61 (s, 0.6H), 5.46 (s, 0.4H), 4.23 (q, J = 6.9 Hz, 0.5H), 3.87 (q, J = 8.7 Hz, 0.5H), 3.25-3.39 (m, 1H), 3.03-3.16 (m, 1H); MS (ESI) *m/z* 222 (M- 1).

(2*RS*, 4*R*)-2-Biphenyl-4-yl-thiazolidine-4-carboxylic acid (2v). Yield (0.66 g, 95%). ^{1}H NMR (DMSO-d_6) δ 7.33-7.68 (m, 10H), 5.74 (s, 0.6H), 5.55 (s, 0.4H), 4.23 (t, J = 6.9 Hz, 0.6H), 3.91 (t, J = 8.4 Hz, 0.4H), 3.29-3.47 (m, 1H), 3.06-3.17 (m, 1H); MS (ESI) *m/z* 284 (M-1).