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## ABSTRACT

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 cancer cells respectively. These agents were originally designed as part of a series of compounds to inhibit lysophosphatidic acid (LPA), a phospholipid growth factor. After discovering the antiproliferation activity of SAP and SDAP in prostate cancer cell lines we propose to synthesize 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 synthetic 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 pharmacophore 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 earlier studies it appeared in our Serine Amide Phosphate (SAP) series that the aliphatic chain is optimum at C-14 while with the new compounds it appears 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 compounds as 2-arylthiazolidine-4-carboxylic acid amides (ATCAAs). This report shares the critical structure activity relationships for optimum activity in prostate cancer cells.

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**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 cancer cells respectively. These agents were originally designed as part of a series of compounds to inhibit lysophosphatidic acid (LPA), a phospholipid growth factor. After discovering the antiproliferation activity of SAP and SDAP in prostate cancer cell lines we propose to synthesize a focused set of SAP and SDAP analogs using the combinatorial parallel-compound solution phase syntheses when appropriate, 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 pharmacophore and allow for the optimization of the antiproliferative actions of this set of drugs.

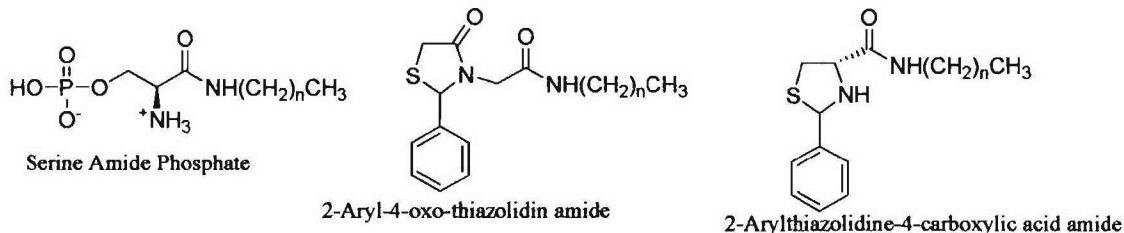
More recently we have discovered a new set of 2-arylthiazolidine-4-carboxylic acid amides that show sub micromolar anticancer activity in the cell lines described above. We have found new synthetic schemes for these new compounds and have expanded our structure activity relationships into the substitutions 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 have been carried forward. 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 found a new set of compounds, the 2-arylthiazolidine-4-carboxylic acid amides (ATCAAs) that have potential for prostate cancer.

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

*Year 4: We will take the advantage of biological studies in year 1-3 to design new generation of analogs in order to optimize the inhibition of proliferation of prostate cancer cells.*

This task was successfully completed. In year 2 we described design, synthesis, and biological evaluation of a new series of 2-aryl-4-oxothiazolidin-3-yl amides in which 4-thiazolidine 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 analogues in terms of cytotoxicity and selectivity, we made closely related structural modifications, which led us to the discovery of a new class of 2-arylthiazolidine-4-carboxylic acid amides (ATCAAs). The detailed structure activity relationship studies of this 3<sup>rd</sup> generation compounds was reported in year 3 report. These compounds were potent cytotoxic agents with IC<sub>50</sub> values in the low micromolar concentration range and demonstrated enhanced selectivity in receptor-negative cells compared to serine amide phosphates (SAPs) and 4-thiazolidinone amides (manuscript 1).



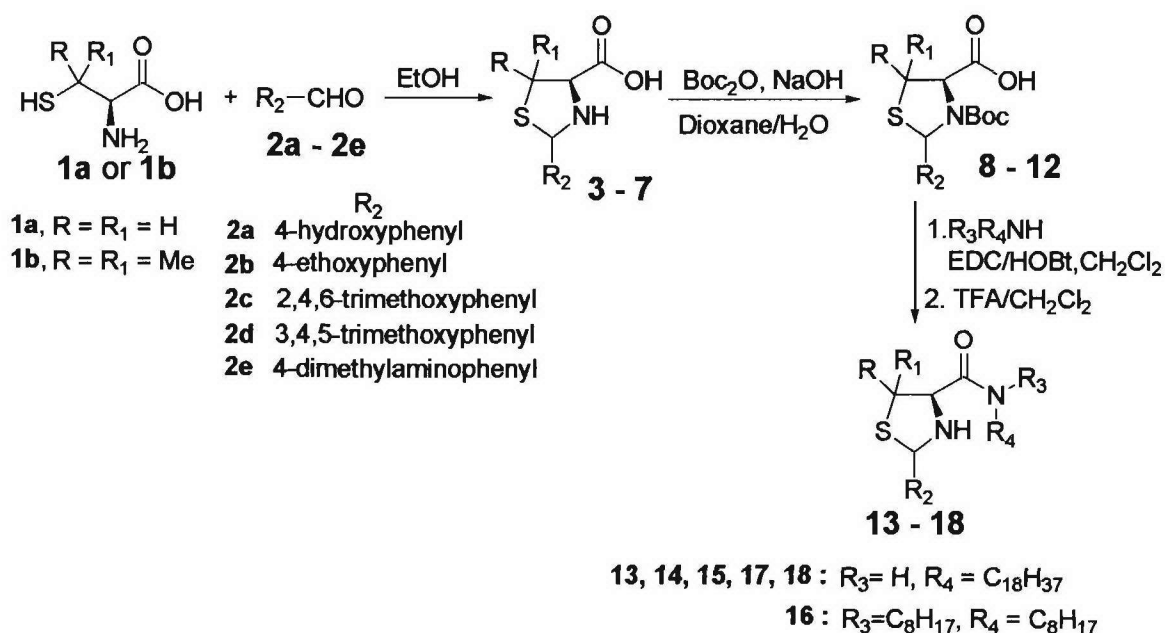
Apoptosis represents a general and delicately efficient cellular suicide pathway. Most of the presently available cytotoxic anticancer drugs mediate their effect via apoptosis induction in cancer cells. Apoptosis is suggested as one of the major mechanisms for targeted therapy of various cancers including prostate cancer. However, cancer cells become resistant to apoptosis in case of advanced prostate cancer and do not respond to cytotoxic chemotherapeutic agents. Thus, agents that induce apoptotic death of hormone-refractory prostate cancer cells could be useful for the treatment of this malignancy.

Recently, we showed that ATCAAs induce apoptosis in LNCaP and PC-3 cells (manuscript 1). Therefore, we hypothesize that ATCAAs represent a novel class of anti-prostate cancer agents, which were very effective in the inhibition of growth of human prostate cancer cell lines and capable of inducing apoptosis. To further understand the structural features and their anticancer activity, we proposed synthetic optimization of ATCAAs toward potency and selectivity.

The details of this work have been recently published in *Bioorganic & Medicinal Chemistry Letters* (manuscript 2).

The general synthesis of various analogs is shown in Scheme 1. Accordingly, L-cysteine (**1a**) or L-penicillamine (**1b**) was allowed to react with appropriate benzaldehydes (**2a-2e**) in ethanol at ambient temperature to give cyclized products (**3-7**), which were converted to the corresponding Boc derivatives **8-12** as shown in Scheme 1. Reaction of Boc-protected carboxylic acids **8-12** with octadecyl or di-n-octyl amine using EDC/HOBt gave corresponding amides, which were treated with TFA to form the target compounds **13-18**. All new compounds were fully characterized by <sup>1</sup>HNMR, <sup>13</sup>CNMR, IR and Mass spectrometry, in certain cases by elemental analysis.

Scheme 1



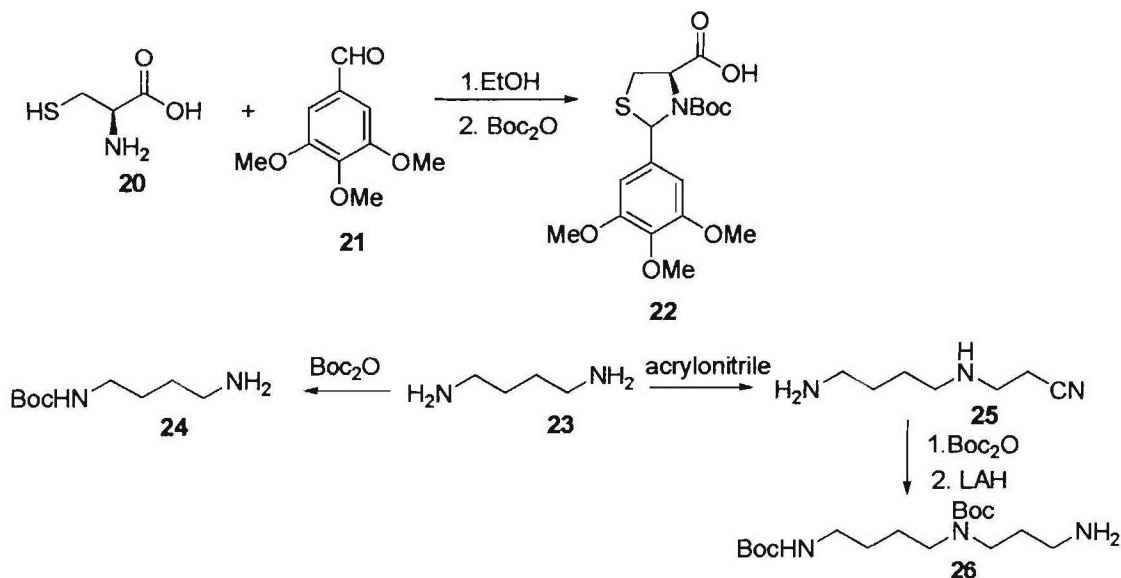
Cancer-bearing animals have elevated levels of polyamines in their extracellular fluids. The potential usefulness of polyamine analogs as antiproliferative agents against many tumor cell lines has been extensively discussed. Prostate gland is a uniquely rich factory of polyamine production. The semen of healthy men contains large amounts of spermine that originates mainly from prostatic secretion. No other human organ has such high polyamine concentrations. Therefore, targeting prostatic polyamines has been a tempting approach for the therapy of prostatic carcinoma.

In year 2 we designed and synthesized serine-polyamine and thiazolidinone-polyamine conjugates. The antiproliferative effects of synthesized compounds were assessed against five human prostate cancer cell lines DU-145, PC-3, LNCaP, PPC-1, and TSU-Pr1 using sulforhodamine B (SRB) assay.

Interestingly, the thiazolidinone-spermine conjugate (**19**) showed enhanced selective antiproliferative activity in prostate cancer cell lines over non-tumor RH7777 cells. Encouraged with these results and in our continued efforts to further optimize this set of compounds for selective potency and to improve their pharmacokinetic properties, we designed a new series of compounds containing thiazolidine-4-carboxylic acid as head group conjugated with naturally occurring polyamines. Initially, we utilized putrescine, spermidine, and spermine polyamines for this study.

Carboxylic acid **22** was synthesized following a method, reported earlier from our laboratory. Treatment of excess of 1,4-diaminobutane with di-*t*-butyl-dicarbonate in chloroform under dilute conditions gave mono protected putrescine (**24**). Reaction of acrylonitrile with 1,4-diaminobutane in methanol gave the adduct which was converted to Boc-protected spermidine (**26**) in two steps as shown in Scheme 2. Reaction of carboxylic acid **22** with protected polyamines (**24** and **26**) in the presence of EDC/HOBt followed by treatment with HCl gave compounds **29** and **30** (Scheme 2).

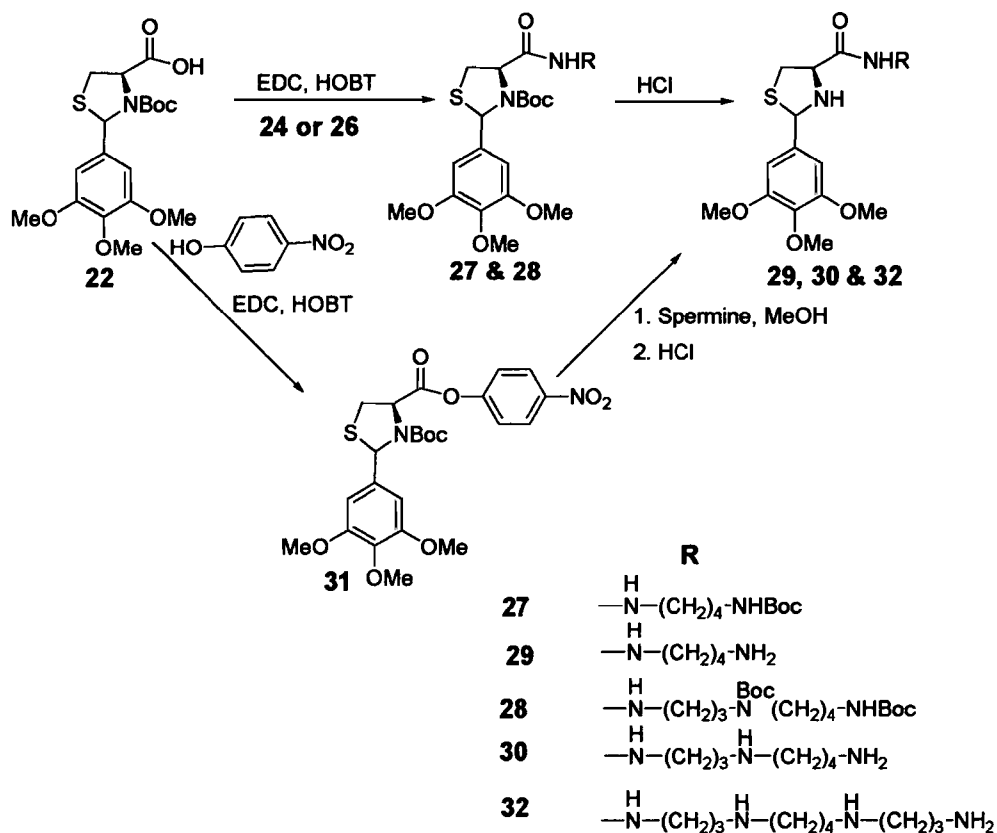
Scheme 2



We adopted a different protocol for the synthesis of spermine conjugates. Firstly, carboxylic acid **22** was converted to corresponding active ester with 4-nitrophenol. Reaction of this active ester **31** with spermine in methanol at ambient temperature gave the corresponding spermine conjugate which was treated with HCl/ $\text{Et}_2\text{O}$  to form the compound **32** (Scheme 3).



Scheme 3

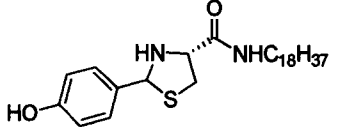
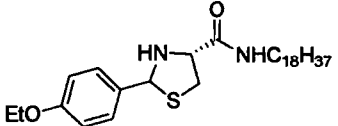
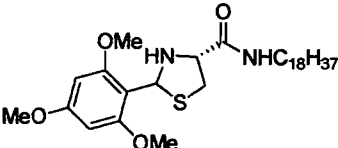
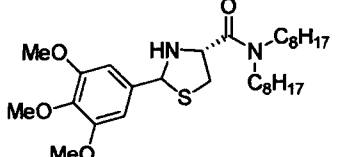
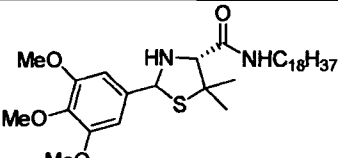
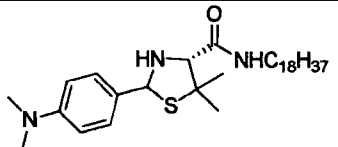


**Task 2. Determine activity of SAP and SDAP analogs in Prostate cell lines**

*Year 4: We will determine the activity of the synthesized analogs in PC-3, DU-145 and LNCaP cell lines.*

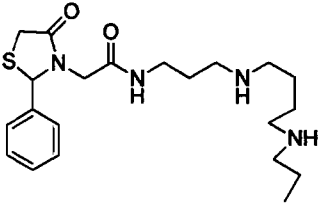
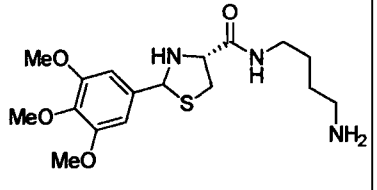
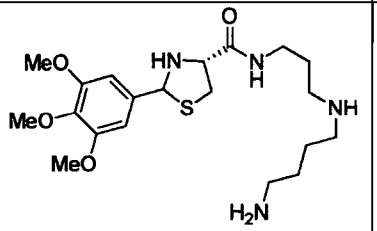
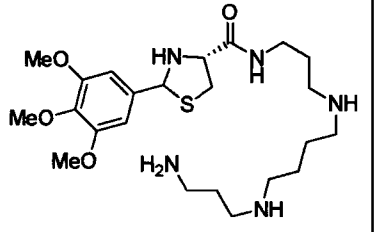
This task was completed successfully. We have tested the cytotoxicity of the synthesized compounds in PC-3, DU-145 and LNCaP prostate cancer cell lines as proposed earlier. In addition to these cell lines we have also tested in two additional PPC-1 and TSU-Pr1 prostate cancer cell lines. To determine the selectivity of these compounds we have also tested them in non-prostate cancer cells RH7777 cells (data shown in Tables 1 & 2).

**Table 1.** Antiproliferative effects of synthesized analogs

ID	Structure	IC <sub>50</sub> (μM)					
		RH 7777 <sup>a</sup>	DU-145 <sup>b</sup>	PC-3 <sup>b</sup>	LNCaP <sup>b</sup>	PPC-1 <sup>b</sup>	TSU-Pr1 <sup>b</sup>
13		5.4	3.1	5.3	1.6	0.82	3.0
14		>20	>20	>20	6.1	4.4	>20
15		>20	7.3	10.6	2.4	0.83	6.1
16		>20	>20	>20	8.7	14.1	>20
17		>20	>20	>20	>20	18.8	>20
18		>20	>20	>20	>20	>20	>20
<b>5-Fluorourasil</b>		ND <sup>c</sup>	11.9	12.0	4.9	6.4	3.6

<sup>a</sup>Control cell line. <sup>b</sup>Prostate cancer cell lines. <sup>c</sup>ND = Not determined.

**Table 2.** Antiproliferative effects of polyamine conjugates

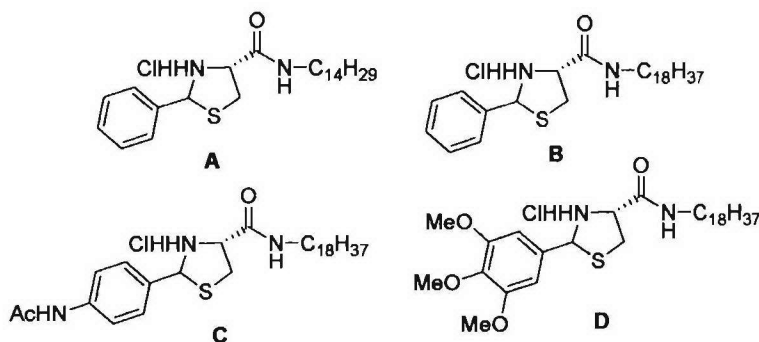
ID	Structure	IC <sub>50</sub> (μM)						
		RH 7777 <sup>a</sup>	DU-145 <sup>b</sup>	PC-3 <sup>b</sup>	LNCa-p <sup>b</sup>	PPC-1 <sup>b</sup>	TSU-Pr1 <sup>b</sup>	MCF-7 <sup>c</sup>
19.HCl		68.4	5.3	7.0	12.2	5.7	3.2	>100
29.HCl		>100	>100	>100	>100	>100	>100	>100
30.HCl		>100	>100	>100	>100	>100	>100	>100
32.HCl		71.9	5.1	5.9	10.9	5.0	3.0	>100

<sup>a</sup>Control cell line. <sup>b</sup>Prostate cancer cell lines. <sup>c</sup>Breast cancer cell line.

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

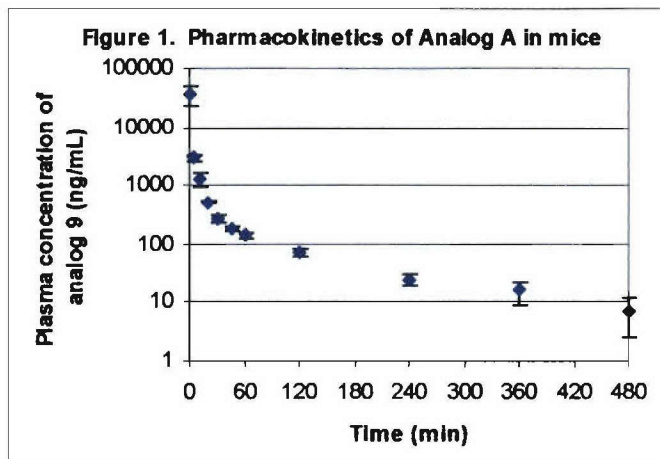
Year 4: We will select the most promising agents from specific Aim.6 of the PC-3, DU-145 and LNCaP cell lines studied in year 1, 2 and 3 for In Vitro Efficacy against Prostate Tumor Xenografts in mice (Specific Aim C.7)

Before assessing in vivo activities of these thiazolidine analogs (ATCAAs), we first tested the acute (30 day) toxicity and pharmacokinetics of selected thiazolidines. Daily subcutaneous doses (10 mg/kg of analog A, 5 mg/kg of analog B, 10 mg/kg of analog C, or 10 mg/kg of analog D) did not produce any signs of toxicity as demonstrated by lack of body weight loss. We developed and validated an LC/MS bioanalytical method for quantitation of drug concentrations in mouse plasma. Briefly, plasma proteins were precipitated with acetonitrile, centrifuged, and the supernatant fraction directly injected to the LC/MS. Analytes were detected using selected ion monitoring in the positive-ion mode using electrospray ionization and an Agilent 1100 coupled to a single quadrupole mass spectrometer.

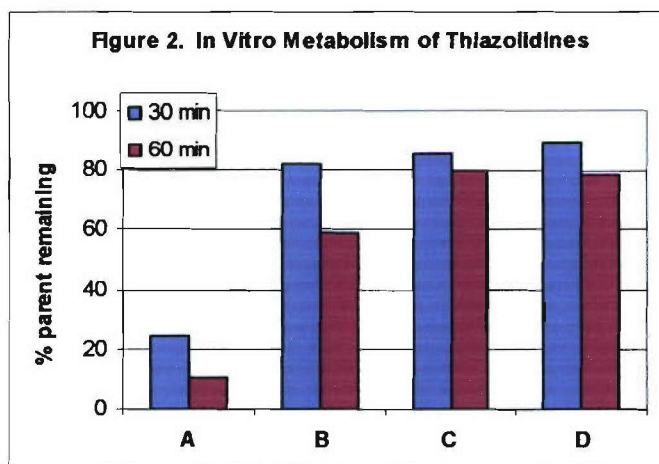


We then examined the pharmacokinetics of analog A in ICR mice after a 10 mg/kg intravenous dose (Figure 1). Compound A demonstrated moderate clearance (56 mL/min/kg) and distribution (volume of distribution was 1.3 L/kg), with a terminal half-life of 2 h. The relatively short in vivo half-life of analog A in mice prompted us to examine the in vitro hepatic metabolism of several lead compounds (i.e., analogs A-D; Figure 2). Analog A with a shorter (C14) alkyl chain was metabolized more rapidly in vitro compared to B with a C18 alkyl chain. Thiazolidines C and D with 2-aryl ring substituents were most stable during in vitro metabolism studies.

Hydroxylation of the alkyl chain was a major metabolic modification found in A, with the subsequent formation of a carboxylic acid metabolite, suggesting that alkyl chain length is also an



important determinant of in vivo metabolic stability and pharmacokinetics. Importantly, the structural modifications that enhanced in vitro cytotoxicity also enhanced in vitro metabolic stability.



### Key Research Accomplishments

- Investigated the effect of position of the substituents and nature of the ether linkage on phenyl head group of ATCAAs.
- Optimized the substitution pattern of methoxy groups on the phenyl head group by synthesizing new analogs. Cytotoxicity data shows that 3,4,5-trimethoxyphenyl analog was more active than corresponding 4-methoxy, 3,4-dimethoxy, and 2,4,6-trimethoxyphenyl derivatives.
- Investigated the significance of amide group in ATCAAs by replacing the amide hydrogen with an alkyl group to provide branched amide **16**, which failed to demonstrate cytotoxicity at concentration below 20  $\mu\text{M}$  in three prostate cancer cell lines except LNCaP and PPC-1 cells.
- Observed that central thiazolidine core in ATCAAs with two chiral centers plays an important role in providing potency and selectivity as simple structural modification by dimethyl substitution at C-5 position lead to decreased potency in all five human prostate cancer cell lines.
- Cytotoxicity data demonstrated that ATCAAs are sensitive to simple modifications or changes, which allowed us to understand the minimum structural requirements of this class

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

- of compounds to exhibit potent and selective anticancer activity against prostate cancer cells.
- f) Synthesized new series of polyamine conjugates containing thiazolidine-4-carboxylic acid as head group conjugated with naturally occurring polyamines. In this small series of compounds, spermine conjugates were found to be most active with enhanced selectivity against prostate cancer cell lines and interestingly they did not show any cytotoxicity in MCF-7 breast cancer cells below 100  $\mu$ M.
  - g) Toxicity and pharmacokinetics of selected ATCAAs indicates that these compounds did not produce any signs of toxicity as demonstrated by lack of body weight loss.
  - h) We developed and validated an LC/MS bioanalytical method for quantitation of drug concentrations (ATCAAs) in mouse plasma.
  - i) Identified metabolic sites of selected thiazolidines by in vitro hepatic metabolism studies.

#### **Reportable Outcomes (Copies attached)**

1. 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, *J. Med. Chem* 2005, 48, 2584-2588.
2. SAR studies of 2-arylthiazolidine-4-carboxylic acid amides: A novel class of cytotoxic agents for prostate cancer.  
Veeresa Gududuru, Eunju Hurh, Joshua Sullivan, James T. Dalton, and Duane D. Miller, *Bioorganic & Medicinal Chemistry Letters*, 2005, 15, 4010-4013.
3. Polyamine Conjugates of Serine, 4-Thiazolidinone and Thiazolidine-4-carboxylic acid: Synthesis and Growth Inhibitory Effects on Human Prostate Cancer Cell Lines.  
Veeresa Gududuru, Eunju Hurh, James T. Dalton, and Duane D. Miller, MEDI, 229<sup>th</sup> ACS National Meeting, San Diego, CA, March 12, 2005.

## Discovery of 2-Arylthiazolidine-4-carboxylic Acid Amides as a New Class of Cytotoxic Agents for Prostate Cancer<sup>†</sup>

Veeresa Gududuru,<sup>‡</sup> Eunju Hurh,<sup>§</sup> James T. Dalton,<sup>§</sup> and Duane D. Miller<sup>\*,‡</sup>

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To improve the selectivity and antiproliferative activity of previously reported serine amide phosphates (SAPs), we designed a new series of 4-thiazolidinone amides, 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 analogues in terms of cytotoxicity and selectivity, we made closely related structural modifications, 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<sub>50</sub> 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).<sup>1–4</sup> 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.<sup>5</sup> 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.<sup>6</sup> Further, prior studies have shown that LPA is mitogenic in prostate cancer cells and that PC-3 and DU-145 cells express LPA<sub>1</sub>, LPA<sub>2</sub>, and LPA<sub>3</sub> receptors.<sup>7</sup> Advanced prostate cancers express LPL receptors and depend on phosphatidylinositol 3-kinase (PI3K) signaling for growth and progression to androgen independence.<sup>2</sup> Thus, these pathways are widely viewed as one of the most promising new approaches to cancer therapy<sup>8</sup> and provide an especially novel approach to the treatment of advanced, androgen-refractory prostate

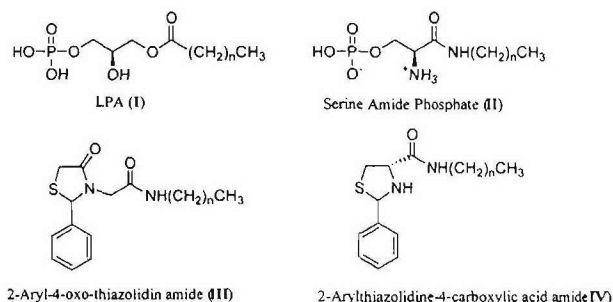


Figure 1.

cancer. Despite the promise of this approach, there are no clinically available therapies that selectively exploit or inhibit LPA or PI3K signaling.

In a previous contribution from our laboratory,<sup>9</sup> we showed that effective cytotoxic agents were obtained, by replacing the glycerol backbone in LPA with serine amide. However, the most potent compounds in that series of derivatives were nonselective and potently killed both prostate cancer and control cell lines. To improve the selectivity and enhance the pharmacokinetic and antiproliferative properties, 2-aryl-4-oxo-thiazolidinone amides with general structure III (Figure 1) were designed, utilizing 4-thiazolidinone moiety as a biomimetic replacement for the phosphate group.<sup>10</sup> This strategic modification showed that the 2-aryl-thiazolidinone moiety is indeed quite beneficial for obtaining a new set of antiproliferative compounds with improved selectivity, but resulted in decreased potency compared to serine amide phosphates.<sup>9</sup> 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-thiazolidinone 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.

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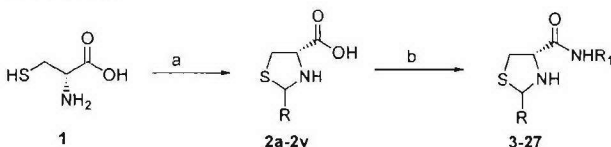
<sup>†</sup> Part of this work was presented at 227th ACS National Meeting, Anaheim, CA, March 28–April 1, 2004 and at 95th AACR Annual Meeting, Orlando, FL, March 27–March 31, 2004.

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Table 1. Structures and Physical Data of Synthesized Compounds

intermediate 2a-v R	compd	R	R <sub>1</sub>	R <sub>2</sub>	mp (°C)	yield (%)	formula	anal.
phenyl	3·HCl	phenyl	H	C <sub>7</sub> H <sub>15</sub>	ND	80	C <sub>17</sub> H <sub>27</sub> ClN <sub>2</sub> O <sub>2</sub> S	C, H, N
n-dodecyl	4·HCl	phenyl	H	C <sub>14</sub> H <sub>29</sub>	95	83	C <sub>24</sub> H <sub>41</sub> ClN <sub>2</sub> O <sub>2</sub> S	C, H, N
cyclohexyl	5·HCl	phenyl	H	C <sub>18</sub> H <sub>37</sub>	93	70	C <sub>28</sub> H <sub>49</sub> ClN <sub>2</sub> O <sub>2</sub> S	C, H, N
benzyl	6·HCl	phenyl	H	C <sub>19</sub> H <sub>39</sub>	85	78	C <sub>29</sub> H <sub>51</sub> ClN <sub>2</sub> O <sub>2</sub> S	C, H, N
3-indolyl	7	n-dodecyl	H	C <sub>18</sub> H <sub>37</sub>	86	69	C <sub>34</sub> H <sub>68</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N
3-pyridinyl	8	cyclohexyl	H	C <sub>18</sub> H <sub>37</sub>	60	75	C <sub>28</sub> H <sub>54</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N
3-furanyl	9	benzyl	H	C <sub>18</sub> H <sub>37</sub>	80	81	C <sub>29</sub> H <sub>50</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N
4-dimethyl amino phenyl	10	3-indolyl	H	C <sub>18</sub> H <sub>37</sub>	125	65	C <sub>30</sub> H <sub>49</sub> N <sub>3</sub> O <sub>2</sub> S	C, H, N
3-hydroxyphenyl	11	3-pyridinyl	H	C <sub>18</sub> H <sub>37</sub>	94	63	C <sub>27</sub> H <sub>47</sub> N <sub>3</sub> O <sub>2</sub> S	C, H, N
4-methoxyphenyl	12·HCl	3-furanyl	H	C <sub>18</sub> H <sub>37</sub>	99	60	C <sub>26</sub> H <sub>47</sub> ClN <sub>2</sub> O <sub>2</sub> S	C, H, N
3,4-dimethoxyphenyl	13	4-dimethylaminophenyl	H	C <sub>18</sub> H <sub>37</sub>	75	75	C <sub>30</sub> H <sub>53</sub> N <sub>3</sub> O <sub>2</sub> S	C, H, N
3,4,5-trimethoxyphenyl	14	3-hydroxyphenyl	H	C <sub>18</sub> H <sub>37</sub>	50	69	C <sub>28</sub> H <sub>48</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N
4-acetyl amino phenyl	15·HCl	4-methoxyphenyl	H	C <sub>18</sub> H <sub>37</sub>	95	70	C <sub>29</sub> H <sub>51</sub> ClN <sub>2</sub> O <sub>2</sub> S	C, H, N
4-fluorophenyl	16·HCl	3,4-dimethoxyphenyl	H	C <sub>18</sub> H <sub>37</sub>	103	83	C <sub>30</sub> H <sub>53</sub> ClN <sub>2</sub> O <sub>3</sub> S	C, H, N
4-bromophenyl	17·HCl	3,4,5-trimethoxyphenyl	H	C <sub>18</sub> H <sub>37</sub>	115	70	C <sub>31</sub> H <sub>55</sub> ClN <sub>2</sub> O <sub>4</sub> S	C, H, N
4-nitrophenyl	18·HCl	4-acetylaminophenyl	H	C <sub>18</sub> H <sub>37</sub>	170	63	C <sub>30</sub> H <sub>52</sub> ClN <sub>3</sub> O <sub>2</sub> S	C, H, N
4-cyanophenyl	19	4-fluorophenyl	H	C <sub>18</sub> H <sub>37</sub>	65	73	C <sub>28</sub> H <sub>47</sub> FN <sub>2</sub> O <sub>2</sub> S	C, H, N
3,5-difluorophenyl	20	4-bromophenyl	H	C <sub>18</sub> H <sub>37</sub>	81	77	C <sub>28</sub> H <sub>47</sub> BrN <sub>2</sub> O <sub>2</sub> S	C, H, N
2,6-dichlorophenyl	21	4-nitrophenyl	H	C <sub>18</sub> H <sub>37</sub>	115	60	C <sub>28</sub> H <sub>47</sub> N <sub>3</sub> O <sub>3</sub> S	C, H, N
3-bromo-4-fluorophenyl	22	4-cyanophenyl	H	C <sub>18</sub> H <sub>37</sub>	90	70	C <sub>29</sub> H <sub>47</sub> N <sub>3</sub> O <sub>2</sub> S	C, H, N
4-methylphenyl	23	3,5-difluorophenyl	H	C <sub>18</sub> H <sub>37</sub>	113	70	C <sub>28</sub> H <sub>46</sub> F <sub>2</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N
biphenyl	24	2,6-dichlorophenyl	H	C <sub>18</sub> H <sub>37</sub>	49	80	C <sub>28</sub> H <sub>46</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N
	25	3-bromo-4-fluorophenyl	H	C <sub>18</sub> H <sub>37</sub>	100	78	C <sub>28</sub> H <sub>46</sub> BrFN <sub>2</sub> O <sub>2</sub> S	C, H, N
	26	4-methylphenyl	H	C <sub>18</sub> H <sub>37</sub>	120	73	C <sub>29</sub> H <sub>50</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N
	27·HCl	biphenyl	H	C <sub>18</sub> H <sub>37</sub>	130	70	C <sub>34</sub> H <sub>53</sub> ClN <sub>2</sub> O <sub>2</sub> S	C, H, N
	28	phenyl	COCH <sub>3</sub>	C <sub>18</sub> H <sub>37</sub>	90	95	C <sub>30</sub> H <sub>50</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N
	29	phenyl	SO <sub>2</sub> CH <sub>3</sub>	C <sub>18</sub> H <sub>37</sub>	55	90	C <sub>29</sub> H <sub>50</sub> N <sub>2</sub> O <sub>3</sub> S <sub>2</sub>	C, H, N

Scheme 1<sup>a</sup>

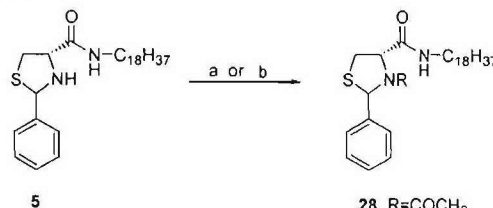
<sup>a</sup> Reagents and conditions: (a) RCHO, EtOH; (b) CH<sub>3</sub>(CH<sub>2</sub>)<sub>n</sub>NH<sub>2</sub>, EDC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>.

## Chemistry

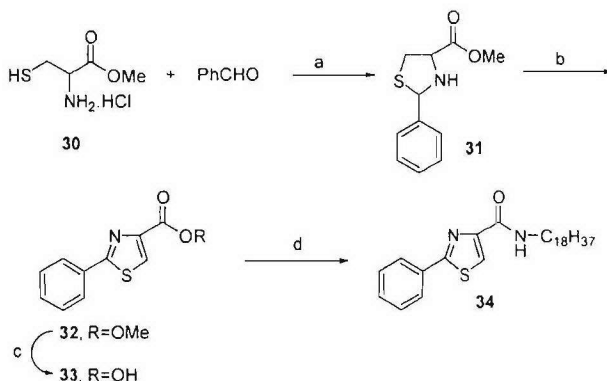
Compounds described in this study were prepared following straightforward chemistry. Reaction of L-cysteine with various aldehydes under reported conditions<sup>11</sup> gave corresponding acids, which were isolated as diastereomeric mixtures. These mixtures were used directly for the formation of corresponding amides by reacting with appropriate alkylamines 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 methyl ester (**30**) 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 (AT-CAAs) 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.<sup>9</sup> We also included a control cell line

Scheme 2<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) Ac<sub>2</sub>O, pyridine; (b) CH<sub>3</sub>SO<sub>2</sub>Cl, pyridine.

Scheme 3<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) NaHCO<sub>3</sub>, EtOH, H<sub>2</sub>O; (b) NBS, CCl<sub>4</sub>; (c) NaOH, MeOH; (d) C<sub>18</sub>H<sub>37</sub>NH<sub>2</sub>, EDC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>.

(RH7777) that does not express LPL receptors,<sup>12</sup> to understand whether the antiproliferative activity of these derivatives was mediated through inhibition of LPL receptors. We first examined LPL receptor expres-



**Table 2.** LPL Receptor mRNA Expression

LPL receptor	old name	expression level relative to $\beta$ -actin					
		RH7777	DU145	PC-3	LNCaP	PPC-1	TSU-Pr1
LPA <sub>1</sub>	EDG-2	UD <sup>a</sup>	2.16	2.53	UD	2.29	2.13
LPA <sub>2</sub>	EDG-4	UD	0.33	0.43	0.32	0.41	0.19
LPA <sub>3</sub>	EDG-7	UD	0.07	0.27	0.28	0.15	UD
sum LPA <sub>1-3</sub>		0	2.56	3.23	0.60	2.85	2.32

<sup>a</sup> UD: under detection limit.**Table 3.** Antiproliferative Effects of Compounds 3–29 and 34

compd	IC <sub>50</sub> ( $\mu$ M)					
	RH7777 <sup>a</sup>	DU-145 <sup>b</sup>	PC-3 <sup>b</sup>	LNCaP <sup>b</sup>	PPC-1 <sup>b</sup>	TSU-Pr1 <sup>b</sup>
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	NA <sup>c</sup>	>20	NA <sup>c</sup>	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 <sup>d</sup>
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
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 <sup>d</sup>	11.9	12.0	4.9	6.4	3.6
paclitaxel	ND <sup>d</sup>	2.7 <sup>e</sup>	3.4 <sup>e</sup>	2.0 <sup>e</sup>	3.4 <sup>e</sup>	2.1 <sup>e</sup>

<sup>a</sup> Control cell line. <sup>b</sup> Prostate cancer cell lines. <sup>c</sup> No activity. <sup>d</sup> Not determined. <sup>e</sup> IC<sub>50</sub> in nM.

sion in these cell lines by RT-PCR to validate their use as in vitro models (Table 2). LPA<sub>1</sub> was the predominant LPL receptor expressed in these cell lines. However, LNCaP cells did not express this receptor subtype. LPA<sub>2</sub> was also expressed in all prostate cancer cell lines examined. Interestingly, ovarian cancer cells also demonstrated overexpression of LPA<sub>2</sub> compared to normal ovarian epithelial cells.<sup>8</sup> PC-3 and LNCaP cells, but not DU-145 cells, expressed LPA<sub>3</sub>, consistent with published data.<sup>7</sup> 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 Table 3. Paclitaxel and 5-fluorouracil were used as reference drugs for comparison. Since preparation of isolated enantiomers was not easy to achieve, the IC<sub>50</sub>s were obtained on diastereomeric mixtures in order to select the most promising compounds. Many of these thiazolidine analogues were very effective in killing prostate cancer cell lines with IC<sub>50</sub> 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<sub>7</sub> to C<sub>18</sub>, 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<sub>14</sub> derivative (4) demonstrated higher potency than 5, but was 8-fold less selective against the RH7777 cell line. Thus, an alkyl chain with C<sub>18</sub> 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.

Replacements of the phenyl ring with a heterocycle, such as an indole, pyridine, or furan ring was investigated by synthesizing analogues 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 analogues. Examination of the IC<sub>50</sub> values of these analogues demonstrates a greater tolerance for diverse substituents in the phenyl ring. In general, the most potent analogues possessed electron-donating substituents

**Table 4.** Thiazolidine Amide-Induced Apoptosis

compd for 72 h		PC-3	LNCaP	RH7777
4	2 $\mu$ M	1.8	14.1	2.6
	5 $\mu$ M	18.7	75.4	3.2
	10 $\mu$ M	54.0	80.7	2.5
5	2 $\mu$ M	1.4	4.5	ND <sup>a</sup>
	5 $\mu$ M	2.3	45.2	
	10 $\mu$ M	3.4	37.1	
	20 $\mu$ M	12.7	26.1	

<sup>a</sup> ND: not determined.

agents, as exemplified by comparison of **13** and **16–18**, relative to **5**. Compound **18** was one of the most active compounds with an IC<sub>50</sub> of 0.55  $\mu$ M and was 38-fold more selective in PPC-1 cells compared to RH7777 cells. On the other hand, thiazolidine analogues (**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. 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  $\mu$ M in both prostate and RH7777 cells, which suggests that thiazolidine ring with two chiral centers plays an important role in providing potency and selectivity.

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 analogues were mediated via apoptotic events. We examined the ability of our analogues to induce apoptosis in LNCaP, PC-3, and RH7777 cells using a quantitative sandwich ELISA<sup>13</sup> that measures DNA–histone complex released during apoptosis. The enrichment factor calculated as ratio of OD405 in treated and untreated 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 analogue **4** was selective in prostate cancer cells despite nonselective cytotoxicity in RH7777 negative control cells (Table 4). Analogue **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. These data suggests that thiazolidine analogues 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 toward the growth of human prostate cancer cell lines. Introduction of ring activating groups on the phenyl ring resulted in increased potencies for prostate cancer cell lines and led to discovery of several new anticancer agents represented by analogues **16**, **17**, and **18** with low/sub micromolar cytotoxicity and high selectivity. From this study, compound **18** emerged as one of the

most potent and selective cytotoxic agents with an IC<sub>50</sub> of 0.55  $\mu$ M and 38-fold selectivity in PPC-1 cells. Further, the ability of these analogues 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 be aimed 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). <sup>1</sup>H NMR spectra were recorded on a Varian 300 instrument. Chemical shifts are reported as  $\delta$  values relative to Me<sub>4</sub>Si 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 2a–v.** A mixture of L-cysteine (1, 0.5 g, 4.12 mmol) and appropriate aldehyde (4.12 mmol) in ethanol (15 mL) was stirred at room temperature for 5 h, and the solid separated was collected, washed with diethyl ether and dried to afford **2a–v**.

**(2R,4R)-2-Phenylthiazolidine-4-carboxylic Acid (2a).** Obtained as colorless crystals (0.82 g, 95%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  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).

**General Procedure for the Preparation of 3–27.** A mixture of appropriate carboxylic acid (**2a–v**, 0.3–0.5 g), EDC (1.25 equiv) and HOBT (1 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (25–50 mL) was stirred for 10 min. To this solution, appropriate alkylamine (1 equiv) was added and stirring continued at room temperature for 6–8 h. Reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (100–150 mL) and sequentially washed with water, sat. NaHCO<sub>3</sub>, and brine and dried over Na<sub>2</sub>SO<sub>4</sub>. 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**, and **27**) were converted to corresponding hydrochlorides using 2 M HCl/Et<sub>2</sub>O.

**(2R,4R)-2-Phenylthiazolidine-4-carboxylic Acid Hep-tylamide Hydrochloride (3-HCl).** <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  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-Phenylthiazolidine-4-carboxylic Acid Methyl Ester (31).** To a solution of DL-cysteine (3 g, 24.76 mmol) in MeOH (50 mL) at 0 °C, SOCl<sub>2</sub> (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 **30** was taken up in aqueous EtOH (1:1, 30 mL), NaHCO<sub>3</sub> (2.28 g, 27.23 mmol) was added, and after 10 min, benzaldehyde (2.5 mL, 24.76 mmol) was added and stirring continued for 3 h. CHCl<sub>3</sub> (200 mL) was added to the reaction mixture, washed with water and brine, and dried (Na<sub>2</sub>SO<sub>4</sub>), and solvent was removed in vacuo. The crude product was purified by column chromatography to afford **31** (4.7 g, 85%). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  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).

This compound was synthesized following a reported procedure.<sup>14</sup> *N*-Bromosuccinamide (2.48 g, 13.9 mmol) and benzoyl peroxide (0.05 g) were added to **31** (1.5 g, 6.7 mmol) dissolved in  $CCl_4$  (70 mL), and the solution was refluxed for 6 h. Solvent was removed in vacuo, and the crude product was purified by column chromatography to afford **32** (0.71 g, 48%). <sup>1</sup>H NMR ( $CDCl_3$ )  $\delta$  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, 1 N NaOH (5 mL) was added and stirred for 2 h. To the reaction mixture, EtOAc (30 mL) was added and acidified with 1 N HCl. Extracted with EtOAc (3  $\times$  50 mL), combined extracts were washed with water and brine and 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**. <sup>1</sup>H NMR ( $CDCl_3$ )  $\delta$  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  $\mu$ g ( $LPA_1$ ) or 1  $\mu$ g ( $LPA_2$  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  $\mu$ M of primers. The following primer pairs were used:  $LPA_1$  forward 5'-GCTCCACACACGGATGAGCAACC-3',  $LPA_1$  reverse 5'-GTGGTCATTGCTGTGAACCTCCAGC-3';  $LPA_2$  forward 5'-CTGCTCAGCCGCTCTATTTG-3',  $LPA_2$  reverse 5'-AGGAGCACCCACAAGTCATCAG-3';  $LPA_3$  forward 5'-CC-ATAGCAACCTGACCAAAAAGAG-3',  $LPA_3$  reverse 5'-TCCT-TGTAGGAGTAGATGATGGGG-3';  $\beta$ -actin forward 5'-GCTC-GTCGTCGACAACGGCTC-3',  $\beta$ -actin reverse 5'-CAAACAT-GATCTGGGTCATCTTCTC-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 s, 60 °C ( $LPA_1$ ) or 58 °C ( $LPA_2$  and  $LPA_3$ ) for 30 s, 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  $\beta$ -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 and stained with 0.4% SRB, and the absorbances at 540 nm were 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 analogues 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.

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**Supporting Information Available:** <sup>1</sup>H NMR (300 MHz) and MS (ESI) characterization data for compounds **2b–v** and **4–29** are available free of charge via the Internet at <http://pubs.acs.org>.

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## SAR studies of 2-arylthiazolidine-4-carboxylic acid amides: A novel class of cytotoxic agents for prostate cancer

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**Abstract**—In our continuing efforts to develop novel chemotherapeutic agents for prostate cancer, recently we reported the discovery of 2-arylthiazolidine-4-carboxylic acid amides (ATCAAs) as a new class of cytotoxic agents. Several of them were very effective in killing specific human prostate cancer cell lines with low/sub-micromolar cytotoxicity and high selectivity against control cells in our sulforhodamine B assay. Encouraged with these preliminary results, we decided to further optimize this new scaffold to enhance the potency and selectivity. Current work describes the synthesis, SAR, and biological evaluation of new compounds for their ability to inhibit the growth of five human prostate cancer cell lines. The cytotoxicity data demonstrated that ATCAAs are sensitive to simple modifications or changes, which allowed us to understand the minimum structural requirements of this class of compounds to exhibit potent and selective anticancer activity against prostate cancer cells.

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Prostate cancer is the most common cancer and is the second leading cause of cancer-related deaths in North America.<sup>1</sup> According to American Cancer Society, approximately 30,000 men will die from prostate cancer in the United States in 2005.<sup>2</sup> One out of nine men over 65 years of age is frequently diagnosed with prostate cancer in the United States.<sup>3</sup> Age and hormone are two known factors influencing the incidence of prostate cancer. Recently, dietary pattern has been identified as a major factor for the difference in prostate cancer incidence between Western and Asian countries.<sup>3–5</sup> Hormonal ablation, the basis of systemic therapy, will invariably fail to control the progression of metastatic prostate cancer in the long run.<sup>6</sup> Patients with advanced or metastatic prostate cancer develop hormone-refractory status that becomes fatal because of the growth of androgen-independent tumor cells and the emergence of tumor clones. Agents that induce apoptosis in metastatic prostate cancer are necessary for the cancer chemotherapy and are urgent for the clinical treatment.

Recent signal transduction research has raised the idea that intracellular signaling mechanisms triggered by extracellular hormonal factors acting through heterotrimeric guanine nucleotide-binding protein (G protein)-coupled receptors (GPCRs) can mediate and sustain prostate cancer pathologic process.<sup>7</sup> Patients with advanced prostate cancer express elevated levels of GPCRs and GPCR ligands, suggesting that the GPCR system is activated in the cancerous gland and may contribute to tumor growth.<sup>8</sup> Importantly, inhibition of G protein signaling attenuates prostate cancer cell growth in animal models.<sup>7</sup> However, the nature of intracellular signaling pathways mediating mitogenic effects of GPCRs in prostate cancer is poorly defined.

Apoptosis represents a general and delicately efficient cellular suicide pathway. Most of the currently available cytotoxic anticancer drugs mediate their effect via apoptosis induction in cancer cells.<sup>9</sup> Apoptosis is suggested as one of the major mechanisms for targeted therapy of various cancers including prostate cancer.<sup>10–12</sup> However, cancer cells become resistant to apoptosis in case of advanced prostate cancer and do not respond to cytotoxic chemotherapeutic agents.<sup>13</sup> Thus, agents that induce apoptotic death of hormone-refractory prostate cancer

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cells could be useful for the treatment of this malignancy.

Recently, we reported the discovery of 2-arylthiazolidine-4-carboxylic acid amides (ATCAAs) as a new class of cytotoxic agents for prostate cancer<sup>14</sup> (Fig. 1). These compounds were obtained as third-generation anticancer agents derived from lysophosphatidic acid (LPA), a small bioactive phospholipid that stimulates cell proliferation, migration, and survival by acting on its cognate GPCRs.<sup>15</sup> Accumulating evidence suggests that LPA's actions are concordant with many of the hallmarks of cancer,<sup>16</sup> indicating an important role for LPA in the initiation or progression of malignant disease. Indeed, LPA levels are significantly increased in malignant effusions, and its receptors (LPA<sub>1/2/3</sub>) are aberrantly expressed in prostate cancer cells.<sup>17</sup> Further, we showed that ATCAAs induce apoptosis in LNCaP and PC-3 cells.<sup>14</sup> Therefore, we hypothesize that ATCAAs represent a novel class of anti-prostate cancer agents, which were very effective in the inhibition of growth of human prostate cancer cell lines and capable of inducing apoptosis. To further understand the structural features and their anticancer activity, we herein propose synthetic optimization of ATCAAs toward potency and selectivity. In this paper, we report the synthesis, structure–activity relationship, and antiproliferative activity of new ATCAAs for prostate cancer.

The general synthesis of target compounds is shown in Scheme 1. Accordingly, L-cysteine (**5a**) or L-penicillamine (**5b**) was allowed to react with appropriate benzaldehydes (**6a–6e**) in ethanol at ambient tempera-

ture to give cyclized products (**7–11**), which were converted to the corresponding Boc derivatives **12–16** as shown in Scheme 1. Reaction of Boc-protected carboxylic acids **12–16** with octadecyl or di-*n*-octyl amine using EDC/HOBt gave corresponding amides, which were treated with TFA to form the target compounds **17–22**. All new compounds<sup>18</sup> were characterized by spectroscopy and, in certain cases, by elemental analysis. The structure and antiproliferative effects of synthesized compounds along with previously reported ATCAAs (for comparison) are listed in Table 1.

The prepared compounds were tested for their potency and selectivity against five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, and TSU-Pr1) and RH7777 cells (control cell line) using the sulforhodamine B assay according to a previously reported procedure.<sup>14</sup> RH7777 cells are rat hepatoma cells that does not express LPL receptors. These cells were used as negative controls to understand whether the antiproliferative activity of ATCAAs was mediated through inhibition of LPL receptors. To validate their use as negative controls, we also examined LPL receptor expression in these cells and showed that none of the LPL receptors were expressed in RH7777 cells by RT-PCR.<sup>14</sup> 5-Fluorouracil (5-FU) was used as a reference drug. Analog **17** containing 4-hydroxyphenyl head group was equally active in all five prostate cancer cell lines, but was not selective compared to **1** (with 3-hydroxyphenyl group) against RH7777 cells. Comparison of the IC<sub>50</sub> values of **2** and **18** suggests that an increase in the alkyl chain length of the ether leads to decreased cytotoxicity. Examination of the cytotoxicity data of ATCAAs suggests that electron-donating substituents on the 2-phenyl ring increases the biological activity, and compound **3** with 3,4,5-trimethoxyphenyl head group emerged as one of the most potent and selective cytotoxic agents from our previous study.<sup>14</sup> It was also observed that 3,4,5-trimethoxyphenyl analog was more active than 3,4-dimethoxy and 4-methoxyphenyl derivatives. To further optimize the substitution pattern of methoxy groups on the phenyl ring, **19** was synthesized which showed a decrease in the potency compared to **3** in all prostate cancer cell lines.

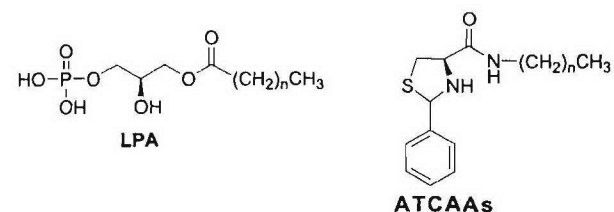
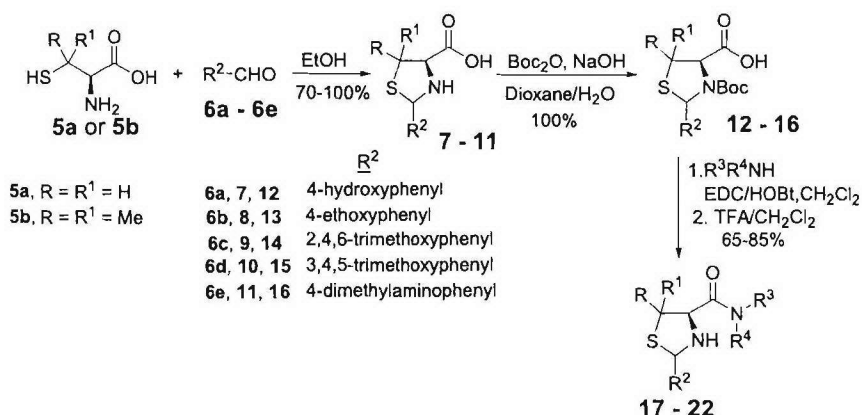


Figure 1.



Scheme 1.

Table 1. Antiproliferative effects of ATCAAs

Compound	Structure	IC <sub>50</sub> (μM)					
		RH7777 <sup>a</sup>	DU-145 <sup>b</sup>	PC-3 <sup>b</sup>	LNCaP <sup>b</sup>	PPC-1 <sup>b</sup>	TSU-Pr1 <sup>b</sup>
1 <sup>c</sup>		31.0	5.7	6.7	1.7	1.2	4.0
2 <sup>c</sup>		>20	8.7	19.0	2.1	1.5	19.6
3 <sup>c</sup>		11.4	3.9	4.0	0.82	0.48	2.4
4 <sup>c</sup>		>20	5.3	6.0	1.6	1.1	3.0
17		5.4	3.1	5.3	1.6	0.82	3.0
18		>20	>20	>20	6.1	4.4	>20
19		>20	7.3	10.6	2.4	0.83	6.1
20		>20	>20	>20	8.7	14.1	>20
21		>20	>20	>20	>20	18.8	>20
22		>20	>20	>20	>20	>20	>20
	5-FU	ND <sup>d</sup>	11.9	12.0	4.9	6.4	3.6

<sup>a</sup> Control cell line.<sup>b</sup> Prostate cancer cell lines.<sup>c</sup> ATCAAs for comparison.<sup>d</sup> ND, not determined.

We showed that ATCAAs have demonstrated chain length (lipophilic side chain)-dependent cytotoxicity with shorter alkyl chain length containing compounds being less active.<sup>14</sup> However, the effect of branching in the lipophilic tail region of ATCAAs on the biological activity was not examined before. To investigate the significance of amide group in ATCAAs, we decided to replace the amide hydrogen with an alkyl group. For these two reasons, compound **20** was synthesized and tested against five human prostate cancer cell lines. Analog **20** failed to demonstrate cytotoxicity at concentration below 20 μM in three prostate cancer

cell lines except LNCaP and PPC-1 cells. Central thiazolidine core in ATCAAs with two chiral centers plays an important role in providing potency and selectivity.<sup>14</sup> We observed that replacement of the thiazolidine ring with more stable thiazole ring resulted in loss of cytotoxicity.<sup>14</sup> Compounds **21** and **22** were prepared to further optimize the central thiazolidine core by dimethyl substitution at C-5 position. However, this simple structural modification did not improve the activity. Indeed, **21** and **22** were active only above 20 μM against all tested five human prostate cancer cell lines.

In conclusion, 2-arylthiazolidine-4-carboxylic acid amides represent a new class of cytotoxic agents for prostate cancer. Furthermore, the anticancer activity of these analogs is attributed to their ability to induce apoptosis in prostate cancer cells. In our continued efforts to optimize ATCAAs toward potency and selectivity, we have prepared and evaluated a new set of compounds for their ability to inhibit the growth of five human prostate cancer cell lines. The SAR study revealed that (1) antiproliferative activity of ATCAAs is sensitive to the position of the substituents on the phenyl ring, (2) introduction of dialkyl (i.e., dioctyl) amide group into the tail region decreases the potency, and (3) modifications to the central thiazolidine core are not favorable. The present data combined with our earlier SAR results provided an insight into the important structural requirements of ATCAAs for their anti-prostate cancer activity. On the basis of these results, we conclude that our next focus will be towards the synthesis of pure stereoisomers of **3** and their pharmacological characterization in animal models, the results of which will be reported in due course.

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- Characteristic data for some compounds are given below. Compound **18**:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.89 (t,  $J = 6.6$  Hz, 3H), 1.27 (s, 32H), 1.4–1.43 (m, 3H), 3.29–3.34 (m, 2H), 3.38–3.41 (m, 1H), 3.71 (dd,  $J = 11.1$ , 3.9 Hz, 1H), 4.01–4.09 (m, 2H), 4.32–4.36 (m, 1H), 5.30 (d,  $J = 12$  Hz, 0.7H), 5.59 (d,  $J = 10.2$  Hz, 0.3H), 6.87–6.92 (m, 2H), 7.39–7.46 (m, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 13.7, 14.4, 22.0, 26.3, 28.6, 28.8, 28.9, 29.0, 31.2, 37.0, 62.8, 65.6, 66.1, 70.6, 71.6, 113.7, 113.9, 128.1, 158.0, 158.3, 169.6, 170.2; MS (ESI)  $m/z$  505 [M+1]. Compound **19**:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.89 (t,  $J = 6.6$  Hz, 3H), 1.26 (s, 32H), 3.12–3.45 (m, 3H), 3.72 (dd,  $J = 7.5$ , 4.5 Hz, 1H), 3.82 (d,  $J = 2.1$  Hz, 3H), 3.84 (s, 6H), 4.14 (br s, 1H), 4.34 (d,  $J = 6$  Hz, 1H), 5.86 (d,  $J = 7.5$  Hz, 1H), 6.16 (d,  $J = 3.9$  Hz, 2H), 7.38 (m, 1H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 13.8, 22.0, 26.1, 26.2, 28.6, 28.9, 31.2, 35.8, 55.2, 55.9, 62.5, 63.8, 66.0, 66.5, 91.5, 105.7, 158.9, 160.7, 169.6, 170.3; MS (ESI)  $m/z$  551 [M+1]. Compound **22**:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.89 (t,  $J = 9$  Hz, 3H), 1.27 (s, 32H), 1.46 (s, 3H), 1.50 (s, 3H), 2.97 (s, 6H), 3.19–3.30 (m, 2H), 3.58 (s, 0.6H), 3.95 (s, 1H, 0.4H), 5.59 (s, 0.5H), 5.64 (s, 0.5H), 6.25 (t,  $J = 6$  Hz, 1H), 6.72 (dd,  $J = 8.7$ , 1.8 Hz, 2H), 7.38–7.43 (m, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.5, 22.1, 25.5, 26.4, 27.2, 27.7, 28.7, 28.8, 28.9, 29.0, 29.1, 31.3, 38.4, 38.7, 39.9, 67.2, 67.9, 73.3, 74.2, 111.8, 111.9, 127.6, 127.9, 168.0, 169.6; MS (ESI)  $m/z$  533 [M+1].

