

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^{*,‡}

Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, Tennessee 38163, and Division of Pharmaceutics, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210

Received September 30, 2004

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 IC₅₀ 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 LPA₁, LPA₂, and LPA₃ 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 therapy⁸ and provide an especially novel approach to the treatment of advanced, androgen-refractory prostate

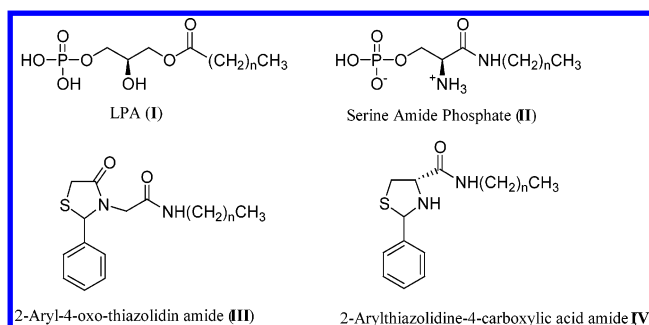


Figure 1.

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

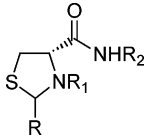
In a previous contribution from our laboratory,⁹ we showed that effective cytotoxic agents were obtained, by replacing the glycerol backbone in LPA with serine amide. However, the most potent compounds in that series of derivatives were nonselective and potently killed both prostate cancer and control 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.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 serine amide phosphates.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-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.

* Corresponding author. 847 Monroe Ave., Rm. 227C, Memphis, TN 38163. Phone: +1-901-448-6026. Fax: +1-901-448-3446. E-mail: dmiller@utmem.edu.

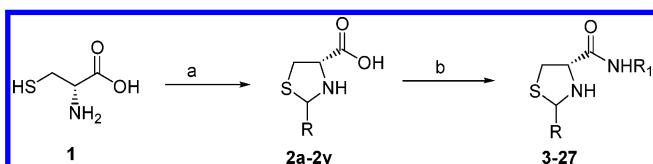
[†] Part of this work was presented at 227th ACS National Meeting, Anaheim, CA, March 28–April 1, 2004 and at 95th AACR Annual Meeting, Orlando, FL, March 27–March 31, 2004.

[‡] University of Tennessee Health Science Center.

[§] The Ohio State University.

Table 1. Structures and Physical Data of Synthesized Compounds


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

Scheme 1^a

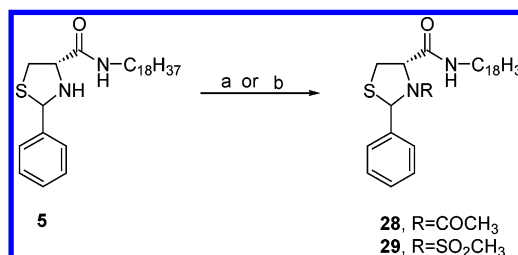
^a Reagents and conditions: (a) RCHO, EtOH; (b) CH₃(CH₂)_nNH₂, EDC, HOBT, CH₂Cl₂.

Chemistry

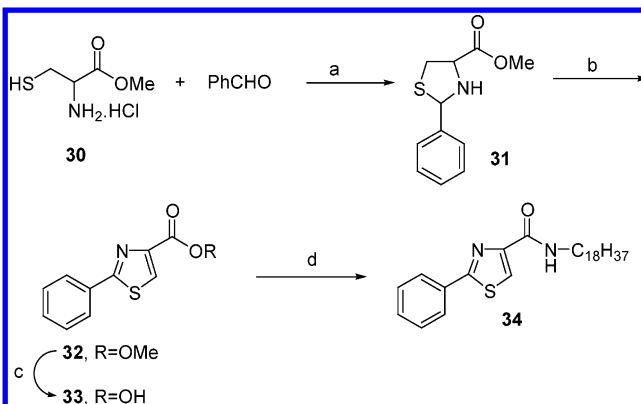
Compounds described in this study were prepared following straightforward chemistry. Reaction of L-cysteine with various aldehydes under reported conditions¹¹ gave corresponding acids, which were isolated as diastereomeric mixtures. These mixtures were used directly for the formation of corresponding 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.⁹ We also included a control cell line

Scheme 2^a

^a Reagents and conditions: (a) Ac₂O, pyridine; (b) CH₃SO₂Cl, pyridine.

Scheme 3^a

^a Reagents and conditions: (a) NaHCO₃, EtOH, H₂O; (b) NBS, CCl₄; (c) NaOH, MeOH; (d) C₁₈H₃₇NH₂, EDC, HOBT, CH₂Cl₂.

(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 expres-

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 **3–29** and **34**

compd	IC ₅₀ (μ M)					
	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	NA ^c	>20	NA ^c	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 ^d
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 ^d	11.9	12.0	4.9	6.4	3.6
paclitaxel	ND ^d	2.7 ^e	3.4 ^e	2.0 ^e	3.4 ^e	2.1 ^e

^a Control cell line. ^b Prostate cancer cell lines. ^c No activity. ^d Not determined. ^e IC₅₀ in nM.

sion in these cell lines by RT-PCR to validate their use as in vitro models (Table 2). LPA₁ was the predominant LPL receptor expressed in these cell lines. However, LNCaP cells did not express this receptor subtype. LPA₂ was also expressed in all prostate cancer cell lines examined. Interestingly, ovarian cancer cells also demonstrated overexpression of LPA₂ compared to normal ovarian epithelial cells.⁸ PC-3 and LNCaP cells, but not DU-145 cells, expressed LPA₃, 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 Table 3. Paclitaxel and 5-fluorouracil were used as reference drugs for comparison. Since preparation of isolated enantiomers was not easy to achieve, the IC₅₀s 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 IC₅₀ values as low as 480 nM (Table 3). Examination of the cytotoxic effects of **3–5** showed that as the chain length increased from C₇ to C₁₈, 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 C₁₄ derivative (**4**) demonstrated higher potency than **5**, but was 8-fold less selective against the RH7777 cell line. Thus, an alkyl chain with C₁₈ 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 IC₅₀ 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 μ M	1.8	14.1	2.6
	5 μ M	18.7	75.4	3.2
	10 μ M	54.0	80.7	2.5
5	2 μ M	1.4	4.5	ND ^a
	5 μ M	2.3	45.2	
	10 μ M	3.4	37.1	
	20 μ M	12.7	26.1	

^a ND: not determined.

uents, as exemplified by comparison of **13** and **16–18**, relative to **5**. Compound **18** was one of the most active compounds with an IC₅₀ of 0.55 μ 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 μ 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 ELISA¹³ 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 IC₅₀ of 0.55 μ 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). ¹H NMR spectra were recorded on a Varian 300 instrument. Chemical shifts are reported as δ values relative to Me₄Si 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%). ¹H 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).

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 CH₂Cl₂ (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 CH₂Cl₂ (100–150 mL) and sequentially washed with water, sat. NaHCO₃, and brine and dried over Na₂SO₄. 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/Et₂O.

(2R,4R)-2-Phenylthiazolidine-4-carboxylic Acid Heptylamide Hydrochloride (3·HCl). ¹H 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-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, SOCl₂ (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), NaHCO₃ (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. CHCl₃ (200 mL) was added to the reaction mixture, washed with water and brine, and dried (Na₂SO₄), and solvent was removed in vacuo. The crude product was purified by column chromatography to afford **31** (4.7 g, 85%). ¹H NMR (CDCl₃) 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).

This compound was synthesized following a reported procedure.¹⁴ *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%). 1H 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, 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**. 1H 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 μ g (LPA_1) or 1 μ 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 μ M of primers. The following primer pairs were used: LPA_1 forward 5'-GCTCCACACACGGATGAGCAACC-3', LPA_1 reverse 5'-GTGGTCATTGCTGTGAACTCCAGC-3'; LPA_2 forward 5'-CTGCTCAGCCGCTCTATTG-3', LPA_2 reverse 5'-AGGAGCACCCACAAGTCATCAG-3'; LPA_3 forward 5'-CCATAGCAACCTGACCAAAAAGAG-3', LPA_3 reverse 5'-TCCT-TGTAGGAGTAGATGATGGGG-3'; β -actin forward 5'-GCTC-GTCGTCGACAACGGCTC-3', β -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 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 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.

Acknowledgment. This research was supported by a grant from the Department of Defense (DAMD17-01-1-083). Pharsight Corporation generously provided WinNonlin software through an Academic License.

Supporting Information Available: 1H 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>.

References

- (1) Raj, G. V.; Barki-Harrington, L.; Kue, P. F.; Daaka, Y. Guanosine phosphate binding protein coupled receptors in prostate cancer: a review. *J. Urol.* **2002**, *167*, 1458–1463.
- (2) Kue, P.; Daaka, Y. Essential role for G proteins in prostate cancer cell growth and signaling. *J. Urol.* **2000**, *164*, 2162–2167.
- (3) Guo, C.; Luttrell, L. M.; Price, D. T. Mitogenic signaling in androgen sensitive and insensitive prostate cancer cell lines. *J. Urol.* **2000**, *163*, 1027–1032.
- (4) Barki-Harrington, L.; Daaka, Y. Bradykinin induced mitogenesis of androgen independent prostate cancer cells. *J. Urol.* **2001**, *165*, 2121–2125.
- (5) Bex, A.; Lummen, G.; Rembrink, K.; Otto, T.; Metz, K.; Rubben, H. Influence of pertussis toxin on local progression and metastasis after orthotopic implantation of the human prostate cancer cell line PC3 in nude mice. *Prostate Cancer Prostatic Dis.* **1999**, *2*, 36–40.
- (6) Qi, C.; Park, J. H.; Gibbs, T. C.; Shirley, D. W.; Bradshaw, C. D.; Ella, K. M.; Meier, K. E. Lysophosphatidic acid stimulates phospholipase D activity and cell proliferation in PC-3 human prostate cancer cells. *J. Cell. Physiol.* **1998**, *174*, 261–272.
- (7) Daaka, Y. Mitogenic action of LPA in prostate. *Biochim Biophys Acta* **2002**, *1582*, 265–269.
- (8) Vivanco, I.; Sawyers, C. L. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat. Rev. Cancer.* **2002**, *2*, 489–501.
- (9) Gududuru, V.; Hurh, E.; Durgam, G. G.; Hong, S. S.; Sardar, V. M.; Xu, H.; Dalton, J. T.; Miller, D. D. Synthesis and biological evaluation of novel cytotoxic phospholipids for prostate cancer. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4919–4923.
- (10) Gududuru, V.; Hurh, E.; Dalton, J. T.; Miller, D. D. Synthesis and Antiproliferative Activity of 2-Aryl-4-oxo-thiazolidin-3-yl-amides for Prostate Cancer. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5289–5293.
- (11) Seki, M.; Mori, Y.; Hatsuda, M.; Yamada, S. A Novel Synthesis of (+)-Biotin from L-Cysteine. *J. Org. Chem.* **2002**, *67*, 5527–5536.
- (12) Svetlov, S.; Sautin, I. Y.; Crawford, J. M. EDG receptors and hepatic pathophysiology of LPA and S1P: EDG-ology of liver injury. *Biochim. Biophys. Acta* **2002**, *1582*, 251–256.
- (13) Huang, H.; Cheville, J. C.; Pan, Y.; Roche, P. C.; Schmidt, L. J.; Tindall, D. J. PTEN induces chemosensitivity in PTEN-mutated prostate cancer cells by suppression of Bcl-2 expression. *J. Biol. Chem.* **2001**, *276*, 38830–38836.
- (14) Badr, M. Z.; Aly, M. M.; Fahmy, A. M.; Mansour, E. Y. Synthesis of Oxazolidines, Thiazolidines, and 5,6,7,8-Tetrahydro-1H, 3H-pyrrolo[1,2-c] oxazole (or thiazole)-1, 3diones from β -Hydroxy- or β -Mercapto- α -amino Acid Esters. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 1844–1847.

JM049208B