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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.

Prostate cancer is the most common cancer and is the second leading cause of cancer-related deaths in North America.[1] According to American Cancer Society, approximately 30,000 men will die from prostate cancer in the United States in 2005.[2] One out of nine men over 65 years of age is frequently diagnosed with prostate cancer in the United States.[3] 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.[3–5] Hormonal ablation, the basis of systemic therapy, will invariably fail to control the progression of metastatic prostate cancer in the long run.[6] 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.[7] 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.[8] Importantly, inhibition of G protein signaling attenuates prostate cancer cell growth in animal models.[7] 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.[9] Apoptosis is suggested as one of the major mechanisms for targeted therapy of various cancers including prostate cancer.[10–12] However, cancer cells become resistant to apoptosis in case of advanced prostate cancer and do not respond to cytotoxic chemotherapeutic agents.[13] 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[14] (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.[15] Accumulating evidence suggests that LPA's actions are concordant with many of the hallmarks of cancer,[16] 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 ($LPA_{1/2/3}$) are aberrantly expressed in prostate cancer cells.[17] Further, we showed that ATCAAs induce apoptosis in LNCaP and PC-3 cells.[14] 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 temperature 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[18] 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.

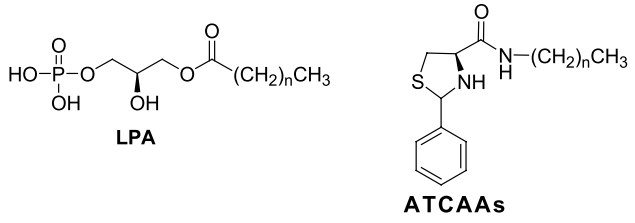
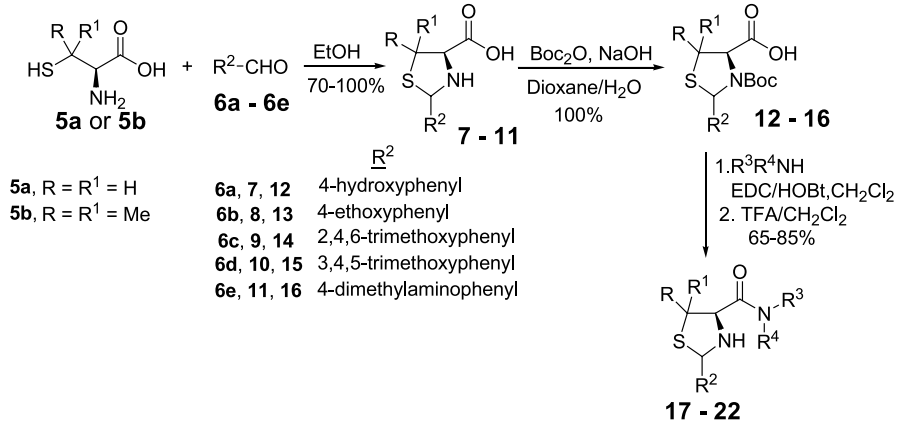


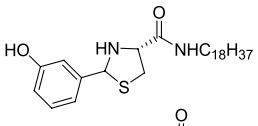
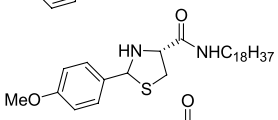
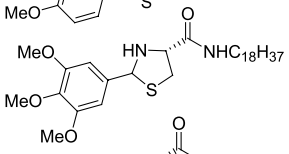
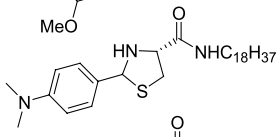
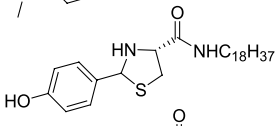
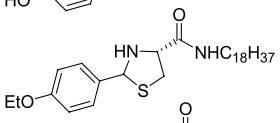
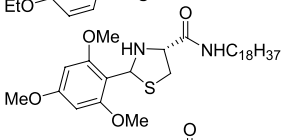
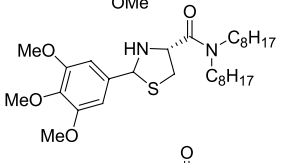
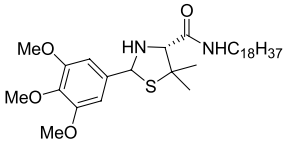
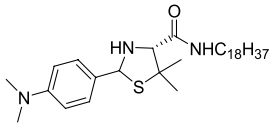
Figure 1.

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.[14] 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.[14] 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_{50} 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.[14] 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.



Scheme 1.

Table 1. Antiproliferative effects of ATCAAs

Compound	Structure	IC_{50} (μM)					
		RH7777[a]	DU-145[b]	PC-3[b]	LNCaP[b]	PPC-1[b]	TSU-Pr1[b]
1[c]		31.0	5.7	6.7	1.7	1.2	4.0
2[c]		>20	8.7	19.0	2.1	1.5	19.6
3[c]		11.4	3.9	4.0	0.82	0.48	2.4
4[c]		>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[d]	11.9	12.0	4.9	6.4	3.6

[a] Control cell line.
[b] Prostate cancer cell lines.
[c] ATCAAs for comparison.
[d] 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.[14] 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.[14] We observed that replacement of the thiazolidine ring with more stable thiazole ring resulted in loss of cytotoxicity.[14] 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.

Acknowledgment

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18. Characteristic data for some compounds are given below. Compound **18**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 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}C NMR (75 MHz, DMSO-d_6) δ: 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}H NMR (300 MHz, $CDCl_3$) δ: 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}C NMR (75 MHz, DMSO-d_6) δ: 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}H NMR (300 MHz, $CDCl_3$) δ: 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, Hz, 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}C NMR (75 MHz, $CDCl_3$) δ: 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].