Published in final edited form as:

Bioorg Med Chem. 2010 January 15; 18(2): 477–495. doi:10.1016/j.bmc.2009.12.020.

Synthesis, in vitro structure-activity relationship, and in vivo studies of 2-arylthiazolidine-4-carboxylic acid amides as anticancer agents

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Abstract

A series of (2*RS*,4*R*)-2-arylthiazolidine-4-carboxylic acid amide (ATCAA) was synthesized. Antiproliferative activity against melanoma and prostate cancer cells compared with control cells (fibroblast and RH7777, respectively) was evaluated. Compound **3id** showed the best selectivity and growth-inhibition activity against three melanoma cell lines (B16-F1, A375, and WM-164). Compounds **15b** and **3ac** had good selectivity and potency against four prostate cancer cell lines (DU 145, PC-3, LNCaP, and PPC-1). The structure-activity relationship (SAR) of the side chain, the thiazolidine ring, and phenyl substituents is discussed. Cell cycle analysis showed that the percentage of cancer cells undergoing apoptosis (sub-G1 phase) increased after treatment with **1b** and **3ad**, which also strongly inhibited melanoma colony formation. In vivo studies on nude mice bearing A375 melanoma tumors showed that compound **1b** inhibited tumor growth in a dose-dependent manner. At a dose of 10 mg/kg, **1b** significantly inhibited melanoma tumor growth and showed higher efficacy than did dacarbazine at 60 mg/kg.

Keywords

Melanoma; Prostate cancer; Thiazolidine; Antiproliferative activity; Structure-activity relationship; Colony formation; *In vivo* evaluation

1. Introduction

Cancer ranks second in diseases leading to mortality, following only cardiovascular diseases. One-quarter of all deaths in the United States are caused by cancers.¹ Out of the many cancer diseases, the incidence of cutaneous malignant melanoma is increasing rapidly throughout the world. Melanoma metastasizing to major organs (stage IV) is virtually

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.12.020.

incurable. Currently, dacarbazine (DTIC) is the only FDA-approved drug to treat advanced melanoma; it provides complete remission in only 2% of patients.^{2–4} Prostate cancer is the most common noncutaneous malignancy in men in Western countries. One out of nine men over 65 years of age is diagnosed with prostate cancer in the United States.⁵ It accounts for one-third of all male cancer diagnoses and 9% of male deaths as a result of cancer.⁶

In previous contributions from our laboratory, we discovered that, by replacing the glycerol backbone in lysophosphatidic acid (LPA, 1-acyl-sn-glycerol-3-phosphate), the resulting 2-arylthiazolidine-4-carboxylic acid amides (ATCAAs) were potent cytotoxic agents for prostate cancer and melanoma.^{7–12} One of earlier derivatives (*2RS,4 R*)-2-phenylthiazolidine-4-carboxylic acid octadecylamide **1a** and (*2RS,4 R*)-2-(4-acetamidophenyl)thiazolidine-4-carboxylic acid hexadecylamide **1b** (Fig. 1) were sent to the U.S. National Cancer Institute 60 human tumor cell line anticancer drug screen (NCI-60). The NCI-60 screening data indicated that compounds **1a** and **1b** strongly inhibited the growth of all nine types of cancer cells with GI₅₀ values ranging from 0.12 μ M (leukemia, CCRF-CEM cell line) to 10.9 μ M (colon cancer, HCC-15 cell line). **1b** was very potent against melanoma (GI₅₀ = 0.13–1.48 μ M against all eight tumor cell lines) and prostate cancer (GI₅₀ = 0.17–0.27 μ M against two tumor cell lines). Based on these preliminary cytotoxic screenings, extensive structure-activity relationship (SAR) and in vivo studies were carried out. In this article, we report the synthesis, biological evaluation, and SAR studies of ATCAA analogues based on **1a** and **1b** for both melanoma and prostate cancer. These analogs were further assessed for their ability to inhibit melanoma colony formation. The in vivo efficacy of compound **1b** was tested in a xenograft model using human A375 melanoma tumors. This class of compounds showed potent antitumor activity and selectivity, which could represent the basis for their development into novel chemotherapeutic drugs.

2. Chemistry

The general synthesis of (*2RS,4 R*)-2-arylthiazolidine-4-carbox-amides is shown in Scheme 1. L-Cysteine reacted with appropriate benzaldehydes in ethanol and water at ambient temperature to give cyclized (*2RS,4 R*)-2-arylthiazolidine-4-carboxylic acids (AT-CAs),¹³ which were converted to the corresponding Boc-protected derivatives **2a–2j**. Reaction of **2a–2j** with appropriate amines using EDCI/HOBt gave corresponding amides, which were subsequently treated with trifluoroacetic acid (TFA) to form the target compounds **1a, 1b**, and **3aa–3jc**. Acetylation of the thiazolidine amino group in compound **1b** with acetyl chloride gave acylation derivative **4**. Ester **5** was obtained by esterifying **2i** with DCC/DMAP catalyzed by (1*S*)-(+)-10-camphorsulfonic acid followed by TFA treatment.¹⁴ Reducing amide **1b** with LAH or B₂H₆ did not provide the desired amine **8b**; instead, disulfanyl dimer **9** was obtained. **8a** and **8b** were prepared from (*2RS,4R*)-2-arylthiazolidine-4-carbal-dehyde **7**, which was obtained from reducing Weinreb amide **6**.¹⁵ The reductive amination of aldehyde **7** with hexadecylamine and NaBH₃CN¹⁶ followed by Boc deprotection gave amines **8a** and **8b**.

The Fmoc-protected (*2RS,4R*)-(4-acetyl-amino-phenyl)-thiazolidine-4-carboxylic acid **10** was prepared by reacting L-cysteine with 4-acetylaminobenzaldehyde, followed by reaction with Fmoc-Cl in the presence of triethanolamine (TEA) with a 99% yield. Subsequent

EDCI/HOBt coupling reaction yielded carboxamide **11**. 4-Aminophenyl intermediate **12** was obtained by acid hydrolysis in methanol. Compound **12** was further reacted with different acid chlorides or sodium cyanate¹⁷ to afford derivatives **14a–14c**. Compounds **13a–13b** and **15a–15c** were obtained by deprotection of the Fmoc group from **12** and **14a–14c**, respectively (Scheme 2).

The open-ring compounds **16a–16d** were prepared as shown in Scheme 3. Compounds **16a–16c** were easily obtained from (*R*)-2-amino-3-(methylthio)propanoic acid or (*R*)-3-(benzylthio)-2-(*tert*-butoxycarbonylamino)propanoic acid. Compound **16d** was readily prepared in a five-step procedure from 2-aminoacetic acid at 38.4% overall yield.

We characterized each compound with nuclear magnetic resonance (NMR), mass spectroscopy, and elemental analysis. Because of the presence of two chiral centers at the thiazolidine ring (C2 and C4 positions, the chirality at C4 is fixed), the ¹H NMR spectra of ATCAAs indicated two diastereomers with a ratio of 7:3. The chemical shifts for C2-H, C4-H, and C5-H_{a/b} on the thiazolidine ring were greatly different between the two diastereomers. For example, in the case of **1b** (Fig. 2A), the C2-H signal of the minor isomer appeared downfield (5.58 ppm in CDCl₃), while that of the major isomer appeared at 5.32 ppm. The C4-H signal of the major isomer appeared at 4.33 ppm, while the minor isomer appeared at 3.90 ppm. The signals from the two nonequivalent protons of C5-H_a and C5-H_b showed four sets of peaks; each set appeared as a doublet of doublet (dd) as a result of geminal coupling and with additional vicinal coupling to C4-H. These peaks were centered at 3.69 ppm, 3.41 ppm, 3.39 ppm, and 3.28 ppm, respectively, with upfield signals partially overlapping with CH₂ signals (3.26–3.21 ppm) from the long fatty chain near the carboxylic amide CONHCH₂C₁₅H₃₁. The absolute structures of the two diastereomers can be readily assigned using 1D nuclear Overhauser effect (NOE) experiments (Fig. 2). When the peak at 4.33 ppm (C4-H of the major isomer) was irradiated, the peak at 5.32 ppm (C2-H of the major isomer) did not show any detectable NOE (Fig. 2B); when the peak at 3.90 ppm (C4-H of the minor isomer) was irradiated, the peak at 5.58 ppm (C2-H of the minor isomer) showed a strong NOE (Fig. 2C). Because the chirality at C4 is fixed (*R*-configuration from starting material L-cysteine), the major isomer was clearly (2*S*,4*R*), and the minor isomer was (2*R*,4*R*) as indicated in Figure 2. The relative distribution of these two configurations can also be expected based on their molecular structures, because it is sterically more favorable when the two bulky groups are attached to opposite sides of the five-membered ring.

3. Biological results and discussion

3.1. Antiproliferative effects of ATCAA against melanoma and prostate cancer

The diastereomeric mixtures of compounds **3aa–15c** were used to evaluate their in vitro inhibitory activity and toxicity against two human melanoma cell lines (A375 and WM-164), one mouse melanoma cell line (B16-F1), four human prostate cancer cell lines (DU 145, PC-3, LNCaP, and PPC-1), fibroblast cells (control cell line for melanoma), and RH7777 (control cell line for prostate cancer). Because preparing pure diastereomers was not easy to achieve, the IC₅₀ values were obtained on diastereomeric mixtures to select the most promising compounds. The standard sulforhodamine B (SRB) assay was used to

evaluate the antiproliferative activity of different compounds in melanoma and prostate cancer cells. The results are summarized in Tables 1 and 2. Compounds **1a** and **1b** were chosen as control compounds for both tumor cell lines in these in vitro studies. Cisplatin (CDDP), DTIC, and Sorafenib (Nexavar, Bay43–9006,¹⁸ which had been granted Fast Track designation by FDA to treat advanced melanoma) were used as reference compounds for melanoma. The antiproliferative data showed that all three melanoma cell lines were resistant to cisplatin. DTIC was inactive because of the lack of bioactivation in vitro.^{19,20} The average IC₅₀ value of Sorafenib on three melanoma cell lines is 5.1 μ M, and the selectivity of Sorafenib between fibroblast cells and melanoma cells is threefold. Our purpose in modifying chemical structure was to enhance the selectivity between tumor cells and control cells and to increase or maintain antitumor potency. Thus, we used the average IC₅₀ value of all melanoma and prostate cancer cells for comparison. Selectivity is defined as the ratio of IC₅₀ values in the control cell line and the average in tumor cell lines. From Tables 1 and 2, the most selective compound (**3id**) had a selectivity of 11.3-fold in melanoma cells against fibroblast cells; compounds **3ac** and **15b** showed an improved selectivity of 9.1-fold and 9.4-fold for prostate cancer cells, respectively.

3.2. Effects of different amide chains, C4 chirality, and chain length of ATCAA

ATCAA molecules were designed from LPA structure, which contains a long aliphatic chain. To examine whether the fatty chain played an essential role in cytotoxicity, we explored the possibilities of mimic fatty chain with bulky ring or aromatic systems such as adamantanyl, fluorenyl, and anthracenyl. Although the anti-proliferative data showed that 1- or 2-adamantanyl amides and 2-benzothiazolylamide (**3ib**, **3ic**, and **3ig**) decreased activity against most melanoma and prostate cancer cells, introducing certain aromatic bulky groups such as 9*H*-fluoren-2-yl (**3id**), anthracen-2-yl (**3ie**), and 4-biphenyl (**3if**) retained their proliferative activity for both types of cancers. Of particular interest, while introducing 9-fluoren-2-yl (**3id**) showed a comparable IC₅₀ value of 2.6 μ M with **1b** (1.8 μ M) on melanoma cells, the selectivity of **3id** increased to 11.3-fold from 8.9-fold in **1b**. This structural modification provided us a new approach to optimize the drug-like properties of ATCAA molecules, in which the hydrophobic fatty lipid chain could be modified with other groups.²¹

To investigate whether the chiral center at the 4-thiazolidine position had an effect on the potency of ATCAA, the 4*S* isomer (**3ai**) was prepared using D-cysteine as starting material. After comparing activity of 4*R* (**3ae**) with 4*S* (**3ai**) isomers in both prostate cancer cell lines and RH7777 cells (Table 4), no significant stereoselectivity at the thiazolidine 4-position was found. Therefore, changing the chirality at C4 on the thiazolidine ring did not substantially affect either activity or selectivity.

Short chain lengths with 8–10 carbon atoms in 4-carboxamide fatty chains (**3ah**, **3ag**) displayed low potency. As chain length increased, potency increased, but toxicity also increased as measured on the RH7777 cell line (Fig. 3). Hexadecyl chains (**3ad**) displayed both the highest potency and selectivity against cancer cells among the examined chains, with an IC₅₀ = 0.4 μ M for PPC-1 cells. However, further increasing chain length (18 carbons, **3aa**) reduced potency and toxicity. Interestingly, adding either a *cis*- or *trans*-double

bond in the C18 side chain restored potency dramatically (*cis* and *trans*-octadec-8-enyl), demonstrating that both length and composition of the side chain are critical for their activity. *trans*-Octadec-8-enyl chain (**3ac**, **3bc**, **3jc**) showed slightly better activity than did the *cis*-isomers (**3ab**, **3bb**, **3jb**) against prostate cancer cells. Introducing a *cis*-double bond in the C16 fatty chain (**3ih**) or an alkyne in the C18 chain (**3ii**) did not increase overall potency on either cancer cell line. Introducing a branched aliphatic chain (**3ia**) decreased the potency in both melanoma cells (average IC₅₀ = 16.7 μM, 9.3-fold decrease) and prostate cancer cells (average IC₅₀ = 2.6 μM, 2.4-fold decrease) compared with **1b** (average IC₅₀ = 1.8 μM for melanoma, 1.1 μM for prostate cancer).

3.3. Effects of substitutions on 2-phenyl of ATCAA

The antiproliferative effects on both melanoma and prostate cancer indicated that when methoxy, 3,4-dimethoxy, and 3,4,5-trimethoxy were introduced to 2-phenyl (**3aa-3ea**), potent activity was preserved or increased compared with that of **1a**. For example, (2*RS*, 4*R*)-2-(3,4,5-trimethoxy-phenyl)thiazolidine-4-carboxylic acid octadecylamide (**3aa**) (average IC₅₀ = 2.9 μM) was 2.4-fold more potent than was **1a** (average IC₅₀ = 6.9 μM) against prostate cancer cells. Subsequently, different groups were introduced to the *para*-phenyl position. Replacements of 4-NHCOCH₃ (**1b**) with 4-N(CH₃)₂ (**3fa**) showed comparable activity on A375 (IC₅₀ = 1.8 vs 2.1 μM) and WM-164 cells (IC₅₀ = 1.5 vs 1.1 μM) but slightly lower selectivity on fibroblast cells (IC₅₀ = 21.0 vs 16.0 μM).

Although both 4-N(CH₃)₂ (**3fa**) and 4-NH₂ (**13b**) substituted AT-CAAs were more potent than was **1a** against prostate cancer cells (average IC₅₀ = 2.8 μM for **3fa**, 1.8 μM for **13b**, and 6.9 μM for **1a**), they were less potent than was **1b** (average IC₅₀ = 1.1 μM and selective ratio = 6.3 for **1b**). Methanesulfonamide (**15a**) or 2-chloroacetamide (**15b**) were less potent (IC₅₀ = 5.7–113.6 μM) and less toxic (IC₅₀ = 25.4–>100 μM) on melanoma cell lines compared with **1b**. Introducing a ureido, 4-NHCONH₂ (**15c**) instead of a 4-NHCOCH₃ kept partial potency (average IC₅₀ = 3.9 μM) but resulted in loss of selectivity against melanoma cells (1.8-fold for **15c** vs 8.9-fold for **1b**). A similar trend with **15a** and **15c** was found in prostate cancer, except that **15b** showed improved growth inhibition of prostate cancer cells (average IC₅₀ of **15b** is 1.0 μM compared with 6.9 μM for **1a** and 1.1 μM for **1b**) and a 9.4-fold selectivity against prostate cancer cells.

We also investigated the importance of *o*-, *m*-, and *p*-substitutions in the 2-phenyl ring relative to acetamino and methoxy groups. Interestingly, *o*-, *m*-, and *p*-isomers showed different activities. *p*-Isomers (**3ea**, **1b**) had the best selectivity and activity for both cancer cell lines. *m*-Isomers (**3da**, **3ha**) had similar average IC₅₀s against melanoma compared with *p*-isomers, but their toxicity also increased on both fibroblast (2.5 μM and 6.3 μM) and RH7777 cells (3.6 μM and 4.9 μM). *o*-MeO and *o*-NHAc analogues (**3ca**, **3ga**) showed slightly less potency and selectivity on both melanoma and prostate cancer cells.

3.4. Effects of 4-linkage of ATCAA and thiazolidine ring

To investigate the importance of amide linkage on C4 of ATCAA, the carboxamide was replaced with an ester and amine. The isosteric replacement of the amide by an ester resulted in **5** with markedly decreased activity against all cancer cell lines. The amine derivatives **8a**

and **8b** also failed to show any increased potency, although they did show lower toxicity (60–84.5 μM) than did **1a** (29.8 μM) and **1b** (16 μM) in fibroblast cells.

Thiazolidine-4-carboxylic acid has been reported to be effective for treating advanced cancers.^{22,23} To examine the importance of a central thiazolidine ring, we synthesized 3-acetyl-thiazolidine compound **4** and a series of thiazolidine ring-opened compounds **16a–16d** as shown in Schemes 1 and 3. Antiproliferative activity of **16a–16d** against melanoma is summarized in Table 3. When acetyl was introduced to 3-NH of thiazolidine, activity decreased in both cancer cell lines (average IC_{50} = 16 and 10.3 μM against melanoma and prostate cancer, respectively). Cleavage of both the C-S and C-N bond (**16a**) led to substantial decrease in both potency (12.9–14.4 μM on melanoma cell lines) and toxicity (61.2 μM on the control cells) compared with **1b** and **3ja** (0.7–2.1 μM on cancer cells, 2.0–16.0 μM on control cells). Opening the thiazolidine ring from the C-S bond (**16b**) caused a loss of potency on both cancer and control cells (>100 μM). Cleavage of the C-N bond in the thiazolidine ring (**16c**) also resulted in decreased activity (12.8–15.1 μM) and no improvement on selectivity compared with **3ja**. Removing the C-S bond (**16d**) reduced the activity of **1b** from IC_{50} = 1.12.1 μM to 10.2–11.9 μM . These results suggested that the presence of the thiazolidine ring is critical for activity. Attempts to reduce the amide with LAH or B_2H_6 resulted in the C-S bond cleavage in thiazolidine. A disulfanyl dimer (**9**), which completely lost anticancer activity, was obtained, and this result is consistent with the above finding that the thiazolidine ring played an essential role in ATCAA potency.

3.5. Flow cytometric analysis

Flow cytometric analysis was performed on human melanoma A375 cells and human prostate cancer LNCaP cells to examine the effect of the new synthetics on cell cycle progression. Analysis of the DNA content of control cells (Fig. 4A–D, top graphs) showed typical distribution of peaks corresponding to cells in G1/G0 phase (65.67–66.97%), S-phase (24.16–28.36%), and G2/M-phase (5.97–8.87%). Upon treatment with compounds **3ad** and **1b** at 1-, 5-, 10-, and 20- μM concentrations for 48 h, flow cytometric analysis showed that these compounds significantly induced apoptosis (sub-G1 phase) in cancer cells in a dose-dependent manner. In both cell lines, the accumulation of cells in sub-G1 phase became apparent at 5 μM (Fig. 4).

3.6. Intracellular calcium mobilization assays of ATCAA

The initial design of ATCAA was derived from contriving mimics of lysophosphatidic acid (LPA), a lipid mediator generated via the regulated breakdown of membrane phospholipids that are known to stimulate G-protein-coupled receptors (GPCR) signaling. GPCR activation could be detected through changes in intracellular calcium concentration. To examine whether ATCAA compounds inhibit cancer cell growth via LPA-GPCR pathway, a fluorescence imaging plate reader FLIPR assay (Ca^{2+} flux response) was conducted to screen **3ad** for dose-dependent agonist and antagonist activities on the LPA1-, LPA3-, and LPA5-transfected Chem-1 cells. Oleoyl-LPA and a known LPA antagonist compound Ki16425²⁴ were used as a positive and negative control, respectively. In agonist assay mode, oleoyl-LPA provided EC_{50} values of 31 nM, 120 nM, and 12 nM for LPA1, LPA3, and LPA5, respectively. When referencing E_{max} of oleoyl-LPA, 15% activation was considered

as an agonist. Compound **3ad** did not exhibit any dose-dependent agonist activity on LPA1, LPA3, or LPA5 (Fig. 5). We also tested antagonism of **3ad**, and 50% inhibition was considered as an antagonist when referencing EC80. The negative control, Ki16425, had an IC₅₀ value of 46 nM on LPA1. However, **3ad** did not exhibit any dose-dependent antagonist activity on the LPA1, LPA3, or LPA5 (Fig. 6). The FLIPR assay results suggested that **3ad** did not display cytotoxicity as a result of antagonism or agonism of the LPA receptors. ATCAA compounds exhibit cytotoxicity through other mechanisms.

3.7. ATCAA inhibits human melanoma colony formation

Cells grown in 96-well plates do not reflect many properties associated with three-dimensional tumors. Human tumor colony formation assay (ex vivo soft agar colony assay) has been suggested as an in vitro method to predict the response of an individual patient's tumor to chemotherapeutic agents.^{25,26} As a prelude to in vivo animal experiments, we investigated the ability of **1b** and **3ad** to inhibit melanoma colony formation by using a well-established method.^{27–29} We performed studies with A375 human melanoma cells. Compounds **1b** and **3ad** could effectively inhibit A375 colony formation at 2 μ M, the lowest tested concentration (Fig. 7). At a concentration of 20 μ M, compound **3ad** almost completely inhibited colony formation, while compound **1b** inhibited colony formation by 75%. At the highest tested concentration, 100 μ M, melanoma colony formation was completely inhibited by both **1b** and **3ad**.

3.8. Antitumor efficacy of ATCAA on human melanoma cancer A375 xenografted athymic nude mice

We chose human melanoma A375 cells to test efficacy in vivo because ATCAA exhibited potency both in vitro assay and colony formation assay against A375 cells. A375 xenograft tumors also exhibited faster tumor growth rate. While compound **3ad** was more potent than was **1b** in inhibiting melanoma colony formation, it had much poorer selectivity between melanoma cancer cells and fibroblast cells (2.0-fold for **3ad** vs 8.9-fold for **1b**) as shown in Table 1. Therefore, we chose **1b** for our in vivo studies against A375 melanoma tumors. ATCAA compound **1b** was examined for in vivo evaluation and was formulated with 80% Tween 80 and 20% Captex 200 because of its limited water solubility.³⁰ We first determined the maximally tolerated dose (MTD) in ICR mice. These mice are less expensive than nude mice and are commonly used for toxicity testing. The MTD for compound **1b** was determined to be 42 mg/kg. Two nontoxic dosages (5 mg/kg and 10 mg/kg) were used. We also included DTIC (60 mg/kg),³¹ the gold standard for melanoma treatment, as a positive control to assess the efficacy of compound **1b**. Briefly, male athymic nude mice were injected subcutaneously with 2.5×10^6 A375 cells. Treatment began on day 7 after tumor inoculation, and **1b** was injected once a day. Each group was composed of eight mice. The results are shown in Figure 8. Compound **1b** at 5 mg/kg showed moderate melanoma tumor growth inhibition. After 22 days of treatment, the percentage of tumor reduction was 25%. At a higher dose of 10 mg/kg, compound **1b** showed significant melanoma tumor growth inhibition with 62% of tumor reduction. Compared with DTIC at a dose of 60 mg/kg, which inhibited tumor growth by 42% at the end of treatment, compound **1b** clearly showed

superior activity in this human melanoma xenograft model. All mice displayed normal activities, and no significant body weight loss was observed during the experiment.

4. Conclusions

We synthesized a series of ATCAA compounds and thiazolidine ring-opened analogues. Chemical modification and structure-activity relationship of ATCAA compounds were investigated with different substituted 2-phenyl, thiazolidine ring, 4-position linkage, and 4-carboxamide groups (Fig. 9) based on biological evaluation against melanoma and prostate cancer cells in vitro. Two compounds, **3id** and **3ac**, and another, **15b**, are promising agents against melanoma and prostate cancer cells, respectively. Compounds **1b** and **3ad** effectively inhibited A375 melanoma colony formation and induced apoptosis. Compound **1b** at 10 mg/kg significantly inhibited melanoma tumor growth in vivo and showed higher efficacy than did DTIC at 60 mg/kg. These compounds did not seem to work via the LPA-GPCR pathway. Additional work is under way to investigate the mechanisms of action of ATCAA and to perform further in vivo studies with other active ATCAA compounds and in prostate cancer xenografted models.

5. Experimental section

5.1. General

All reagents were purchased and used without further purification from Sigma-Aldrich Chemical Co. (St. Louis, MO), Fisher Scientific (Pittsburgh, PA) and AK Scientific, Inc (Mountain View, CA). Moisture-sensitive reactions were conducted in an argon atmosphere. Argon gas was purchased from NexAir Medical Gas, Inc., TN. Routine thin layer chromatography (TLC) was performed on aluminum-backed uniplates (Analtech, Newark, DE). NMR spectra were obtained on a Bruker AX 300 (Billerica, MA) spectrometer. Chemical shifts were reported as parts per million (ppm) relative to TMS in CDCl₃ or DMSO-*d*₆. Mass spectral data were collected on a Bruker ESQUIRE electrospray/ion trap instrument in positive and negative ion modes. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA).

5.2. General procedure for preparing (2*RS*,4*R*)-2-arylthiazolidine-3, 4-dicarboxylic acid 3-*tert*-butyl ester **2a-2j**

A mixture of L-cysteine (3.16 g, 26.11 mmol) and appropriate aldehyde (26.15 mmol) in ethanol (300 mL) and water (30 mL) was stirred at room temperature for 6–15 h, and the separated solid was collected, washed with diethyl ether, and dried to obtain (2*RS*,4*R*)-2-arylthiazolidine-4-carboxylic acid (ATCA) with yields of 70–99%. At 0 °C, ATCA (5.95 mmol) was dissolved in 1 N NaOH (6 mL) and 1,4-dioxane (15 mL); then di-*tert*-butyldicarbonate (2.80 g, 12.80 mmol) was added slowly and stirred at room temperature for 1 h. The reaction mixture was concentrated in a vacuum and washed with ethyl acetate (20 mL). The aqueous phase was adjusted to pH 4 by adding 1 N HCl or 5% KHSO₄, then extracted with ethyl acetate, dried with magnesium sulfate, filtered, and concentrated in a vacuum to give corresponding compounds **2a-2j** as white foam-solids with yields of 89–97%. **2a-2j** were used further in the next step of synthesis without purification.

5.3. General procedure for preparing (2*RS*,4*R*)-2-arylthiazolidine-4-carboxylic acid amide **1a**, **1b**, and **3aa-3jc**

A mixture of appropriate Boc-protected carboxylic acids (**2a-2j**, 0.3–0.5 g), EDCI (1.2 equiv), and HOBT (1.05 equiv) in CH₂Cl₂ (20 mL) was stirred at room temperature for 10 min. To this solution, appropriate amine (1.05 equiv) and Et₃N (1.2 equiv) were added, and stirring continued at room temperature for 6–15 h. The reaction mixture was diluted with CH₂Cl₂ (30 mL) and sequentially washed with water, satd NaHCO₃, and brine and dried over MgSO₄. The solvent was removed under reduced pressure to yield a crude oil, which was stirred with TFA (0.6–1 mL) in 20 mL CH₂Cl₂ at room temperature for 1–8 h to cleave the Boc group. The reaction mixture was concentrated, washed with satd NaHCO₃, and dried over MgSO₄. The solvent was removed to yield a crude solid; ATCAAs **1a-3jc** were purified by silica column flash chromatography using hexane/ethyl acetate gradient elution system. Yield was reported as 2-step yield.

5.3.1. (2*RS*,4*R*)-2-Phenyl-thiazolidine-4-carboxylic acid octadecylamide (**1a**)

—Yield: 76.1%. ¹H NMR (300 MHz, CDCl₃) δ 7.63–7.37 (m, 6H), 5.70 (br, 2H), 5.67 and 5.49 (s, s, 1H, 0.3H and 0.7H), 4.90 and 4.63 (br, t, *J* = 4.5 Hz, 1H), 3.66–3.46 (m, 2H), 3.30–3.26 (m, 2H), 1.54–1.50 (m, 2H), 1.25 (s, 30H), 0.87 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 461.4 [M+H]⁺. Anal. Calcd for C₂₈H₄₈N₂O₃SCF₃COOH: C, 62.69; H, 8.59; N, 4.87. Found: C, 62.94; H, 8.84; N, 4.95.

5.3.2. (2*RS*,4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid hexadecylamide (**1b**)

—Yield: 61.3%. ¹H NMR (300 MHz, CDCl₃) δ 7.54–7.43 (d, d, 4H, *J* = 8.4 Hz), 7.25 (br, 1H), 5.62 and 5.33 (s, s, 0.3H and 0.7H), 4.36 and 4.05 (br, 1H), 3.69 and 3.43 (dd, m, 2H, *J* = 4.2 Hz), 3.31–3.24 (m, 2H), 2.19 (s, 3H), 1.53–1.47 (m, 4H), 1.26 (s, 26H), 0.88 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 490.5 [M+H]⁺, 512.5 [M+Na]⁺, 488.1 [M–H][–]. Anal. Calcd for C₂₈H₄₇N₃O₂S: C, 68.51; H, 9.61; N, 8.48. Found: C, 68.67; H, 9.67; N, 8.58.

5.3.3. (2*RS*,4*R*)-2-(3,4,5-Trimethoxy-phenyl)-thiazolidine-4-carboxylic acid octadecylamide (**3aa**)

—Yield: 65.3%. ¹H NMR (300 MHz, CDCl₃) δ 7.20 and 6.31 (t, 0.7H, *J* = 6.6 Hz and br, 0.3H), 6.77 and 6.71 (s, s, 0.7H and 1.3H), 5.55 and 5.28 (s, s, 0.3H and 0.7H), 4.34 and 3.93 (dd, *J* = 7.8 Hz, 4.2 Hz, m, 1H), 3.88 and 3.87 (s, s, 6H), 3.80 and 3.79 (s, s, 3H), 3.68 and 3.42 (dd, *J* = 11.1 Hz, 4.2 Hz, m, 2H), 3.31–3.19 (m, 2H), 2.52 (br, 1H), 1.50 (m, 2H), 1.25 (s, 30H), 0.87 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 551.5 [M+H]⁺, 573.5 [M+Na]⁺, 549.2 [M–H][–]. Anal. Calcd for C₃₁H₅₄N₂O₄S: C, 67.59; H, 9.88; N, 5.09. Found: C, 67.38; H, 9.95; N, 5.09.

5.3.4. (2*RS*,4*R*)-2-(3,4,5-Trimethoxy-phenyl)-thiazolidine-4-carboxylic acid octadec-8-*cis*-enylamide (**3ab**)

—Yield: 58.8%. ¹H NMR (300 MHz, CDCl₃) δ 7.20 and 6.31 (t, 0.7H, *J* = 6.0 Hz and br, 0.3H), 6.77 and 6.71 (s, s, 0.7H and 1.3H), 5.55 and 5.28 (s, s, 0.3H and 0.7H), 5.37–5.32 (m, 2H), 4.34 and 3.94 (dd, *J* = 7.2 Hz, 4.2 Hz, m, 1H), 3.88 and 3.87 (s, s, 6H), 3.84 and 3.84 (s, s, 3H), 3.68 and 3.43 (dd, *J* = 11.1 Hz, 4.2 Hz, m, 2H), 3.33–3.21 (m, 2H), 2.01–1.97 (br, 4H), 1.97 (br, 1H), 1.55–1.44 (m, 2H), 1.26 (s, 22H), 0.87

(t, 3H, $J=6.9$ Hz). MS (ESI) m/z 549.5 $[M+H]^+$, 547.2 $[M-H]^-$. Anal. Calcd for $C_{31}H_{52}N_2O_4S$: C, 67.84; H, 9.55; N, 5.10. Found: C, 67.49; H, 9.55; N, 5.12.

5.3.5. (2*RS*,4*R*)-2-(3,4,5-Trimethoxy-phenyl)-thiazolidine-4-carboxylic acid octadec-8-*trans*-enylamide (3ac)—Yield: 42.1%. 1H NMR (300 MHz, $CDCl_3$) δ 7.19 and 6.29 (t, 0.7H, $J=6.3$ Hz and br, 0.3H), 6.77 and 6.71 (s, s, 0.7H and 1.3H), 5.55 and 5.28 (s, s, 0.3H and 0.7H), 5.37 (br, 2H), 4.34 and 3.95 (dd, $J=7.8$ Hz, 4.2 Hz, m, 1H), 3.88 and 3.87 (s, s, 6H), 3.84 (s, 3H), 3.68 and 3.43 (dd, $J=11.1$ Hz, 4.2 Hz, m, 2H), 3.31–3.19 (m, 2H), 1.97 (br, 4H), 1.55–1.46 (m, 2H), 1.26 (s, 22H), 0.87 (t, 3H, $J=6.6$ Hz). MS (ESI) m/z 583.2 $[M+Na]^+$, 549.5 $[M+H]^+$, 547.2 $[M-H]^-$. Anal. Calcd for $C_{31}H_{52}N_2O_4S$: C, 67.84; H, 9.55; N, 5.10. Found: C, 68.10; H, 9.62; N, 5.11.

5.3.6. (2*RS*,4*R*)-2-(3,4,5-Trimethoxy-phenyl)-thiazolidine-4-carboxylic acid hexadecylamide (3ad)—Yield: 46.7%. 1H NMR (300 MHz, $CDCl_3$) δ 7.20 and 6.30 (t, 0.7H, $J=6.3$ Hz and br, 0.3H), 6.77 and 6.71 (s, s, 0.7H and 1.3H), 5.55 and 5.28 (d, d, 0.3H, $J=10.2$ Hz and 0.7H, $J=10.8$ Hz), 4.34 and 3.93 (dd, br, m, $J=4.2$ Hz, 1H), 3.89 and 3.88 (s, s, 6H), 3.85 and 3.84 (s, s, 3H), 3.68 and 3.45–3.39 (dd, m, 2H), 3.38–3.19 (m, 2H), 2.63 and 2.52 (br, br, 1H), 1.53 (m, 2H), 1.25 (s, 26H), 0.87 (t, 3H, $J=6.3$ Hz). MS (ESI) m/z 524.1 $[M+H]^+$, 523.8 $[M-H]^-$. Anal. Calcd for $C_{29}H_{50}N_2O_4S$: C, 66.63; H, 9.64; N, 5.36. Found: C, 66.41; H, 9.63; N, 5.30.

5.3.7. (2*RS*,4*R*)-2-(3,4,5-Trimethoxy-phenyl)-thiazolidine-4-carboxylic acid tetradecylamide (3ae)—Yield: 51.0%. 1H NMR (300 MHz, $CDCl_3$) δ 7.20 and 6.27 (t, 0.7H, $J=6.3$ Hz and br, 0.3H), 6.77 and 6.71 (s, s, 0.7H and 1.3H), 5.56 and 5.28 (d, d, $J=10.2$ Hz and 0.7H, $J=10.5$ Hz), 4.34 and 3.93 (dd, $J=4.2$ Hz, m, 1H), 3.89 and 3.88 (s, s, 6H), 3.85 and 3.84 (s, s, 3H), 3.68 and 3.45–3.36 (dd, m, 2H), 3.34–3.19 (m, 2H), 2.51 (br, 1h), 1.50 (m, 2H), 1.25 (s, 22H), 0.87 (t, 3H, $J=6.9$ Hz). MS (ESI) m/z 495.6 $[M+H]^+$. Anal. Calcd for $C_{27}H_{46}N_2O_4S$: C, 65.55; H, 9.37; N, 5.66. Found: C, 65.49; H, 9.41; N, 5.59.

5.3.8. (2*RS*,4*R*)-2-(3,4,5-Trimethoxy-phenyl)-thiazolidine-4-carboxylic acid dodecylamide (3af)—Yield: 63.9%. 1H NMR (300 MHz, $CDCl_3$) δ 7.20 and 6.31 (t, br, 0.7H and 0.3H), 6.77 and 6.71 (s, s, 0.7H and 1.3H), 5.55 and 5.29 (d, d, br, 0.3H, $J=7.8$ Hz and 0.7H, $J=7.5$ Hz), 4.34 and 3.93 (br, m, 1H), 3.89 and 3.88 (s, s, 6H), 3.85 and 3.84 (s, s, 3H), 3.68 and 3.47–3.36 (m, m, 2H), 3.33–3.19 (m, 2H), 2.53 (br, 1H), 1.53 (m, 2H), 1.25 (s, 18H), 0.87 (t, 3H, $J=6.9$ Hz). MS (ESI) m/z 490.3 $[M+Na]^+$. Anal. Calcd for $C_{25}H_{42}N_2O_4S$: C, 64.34; H, 9.07; N, 6.00. Found: C, 64.29; H, 9.06; N, 5.92.

5.3.9. (2*RS*,4*R*)-2-(3,4,5-Trimethoxy-phenyl)-thiazolidine-4-carboxylic acid decylamide (3ag)—Yield: 50.0%. 1H NMR (300 MHz, $CDCl_3$) δ 7.21 and 6.32 (br, br, 0.7H and 0.3H), 6.77 and 6.71 (s, s, 0.7H and 1.3H), 5.55 and 5.28 (s, s, 0.3H and 0.7H), 4.34 and 3.94 (dd, $J=7.8$ Hz and 4.2 Hz, m, 1H), 3.89 and 3.88 (s, s, 6H), 3.85 and 3.84 (s, s, 3H), 3.68 and 3.45–3.36 (dd, m, 2H), 3.34–3.19 (m, 2H), 1.53 (m, 2H), 1.25 (s, 14H), 0.87 (t, 3H, $J=6.9$ Hz). MS (ESI) m/z 461.9 $[M+Na]^+$. Anal. Calcd for $C_{23}H_{38}N_2O_4S$: C, 62.98; H, 8.73; N, 6.39. Found: C, 62.98; H, 8.71; N, 6.28.

5.3.10. (2*RS*,4*R*)-2-(3,4,5-Trimethoxy-phenyl)-thiazolidine-4-carboxylic acid octylamide (3ah)—Yield: 27.7%. ¹H NMR (300 MHz, CDCl₃) δ 7.21 and 6.34 (br, br, 0.7H and 0.3H), 6.77 and 6.71 (s, s, 0.7H and 1.3H), 5.55 and 5.28 (s, s, 0.3H and 0.7H), 4.34 and 3.93 (dd, *J* = 7.8 Hz and 4.2 Hz, m, 1H), 3.89 and 3.88 (s, s, 6H), 3.85 and 3.84 (s, s, 3H), 3.68 and 3.45–3.33 (dd, *J* = 10.8 Hz and 7.8 Hz, m, 2H), 3.31–3.19 (m, 2H), 2.61 (br, 1H), 1.53 (m, 2H), 1.30–1.26 (br, 30H), 0.87 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 411.8 [M + H]⁺, 433.5 [M + Na]⁺. Anal. Calcd for C₂₁H₃₄N₂O₄S: C, 60.11; H, 8.41; N, 6.68. Found: C, 60.36; H, 8.24; N, 6.56.

5.3.11. (2*RS*,4*S*)-2-(3,4,5-Trimethoxy-phenyl)-thiazolidine-4-carboxylic acid tetradecylamide (3ai)—Yield: 49.4%. ¹H NMR (300 MHz, CDCl₃) δ 7.20 and 6.30 (t, br, 0.7H and 0.3H), 6.77 and 6.71 (s, s, 0.7H and 1.3H), 5.56 and 5.28 (s, s, 0.3H and 0.7H), 4.34 and 3.93 (br, m, 1H), 3.89 and 3.88 (s, s, 6H), 3.85 and 3.84 (s, s, 3H), 3.68 and 3.45–3.35 (dd, *J* = 3.9 Hz, m, 2H), 3.34–3.19 (m, 2H), 2.51 (br, 1H), 1.50 (m, 2H), 1.25 (s, 22H), 0.87 (t, 3H, *J* = 6.6 Hz). MS (ESI) *m/z* 495.6 [M + H]⁺. Anal. Calcd for C₂₇H₄₆N₂O₄S: C, 65.55; H, 9.37; N, 5.66. Found: C, 65.29; H, 9.42; N, 5.66.

5.3.12. (2*RS*,4*R*)-2-(3,4-Dimethoxy-phenyl)-thiazolidine-4-carboxylic acid octadecylamide (3ba)—Yield: 29.0%. ¹H NMR (300 MHz, CDCl₃) δ 7.51–6.97 (m, 3H), 6.85 (t, 1H), 5.64 and 5.37 (s, s, 1H), 4.69 and 4.51 (dd, br, m, 1H), 3.91, 3.90, 3.89 and 3.88 (s, s, s, s, 6H), 3.64–3.42 (m, 2H), 3.35–3.23 (m, 2H), 2.52 (br, 1H), 1.51–1.43 (m, 2H), 1.25 (s, 30H), 0.87 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 521.5 [M + H]⁺. Anal. Calcd for C₃₀H₅₂N₂O₄S: C, 69.18; H, 10.06; N, 5.38. Found: C, 69.36; H, 10.01; N, 5.36.

5.3.13. (2*RS*,4*R*)-2-(3,4-Dimethoxy-phenyl)-thiazolidine-4-carboxylic acid (*Z*)-octadec-8-enylamide (3bb)—Yield: 29.1%. ¹H NMR (300 MHz, CDCl₃) δ 7.27 and 6.38 (br, 0.7H and 0.3H), 7.11–6.80 (m, 3H), 5.58 and 5.31 (s, s, 0.3H and 0.7H), 5.39–5.34 (m, 2H), 4.35 and 4.00 (dd, *J* = 7.8 Hz and 4.2 Hz, m, 1H), 3.93, 3.92, 3.90 and 3.89 (s, s, s, s, 6H), 3.70 and 3.44 (dd, *J* = 10.8 Hz and 4.2 Hz, m, 2H), 3.34–3.19 (m, 2H), 2.03–1.99 (br, 4H), 1.56–1.44 (m, 2H), 1.28 (s, 22H), 0.89 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 519.3 [M + H]⁺. Anal. Calcd for C₃₁H₅₀N₂O₄S: C, 69.45; H, 9.71; N, 5.40. Found: C, 69.17; H, 9.61; N, 5.18.

5.3.14. (2*RS*,4*R*)-2-(3,4-Dimethoxy-phenyl)-thiazolidine-4-carboxylic acid (*E*)-octadec-8-enylamide (3bc)—Yield: 13.3%. ¹H NMR (300 MHz, CDCl₃) δ 7.26 and 6.33 (br, 1H), 7.09–6.82 (m, 3H), 5.57 and 5.29 (s, s, 0.3H and 0.7H), 5.37 (br, 2H), 4.34 and 3.95 (dd, *J* = 8.1 Hz and 4.2 Hz, m, 1H), 3.91, 3.90, 3.89 and 3.88 (s, s, s, s, 6H), 3.69 and 3.43 (dd, *J* = 11.1 Hz and 4.2 Hz, m, 2H), 3.33–3.17 (m, 2H), 1.96 (br, 4H), 1.52–1.43 (m, 2H), 1.26 (s, 22H), 0.87 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 519.5 [M + H]⁺, 541.3 [M + Na]⁺, 517.2 [M – H][–]. Anal. Calcd for C₃₁H₅₀N₂O₄S: C, 69.45; H, 9.71; N, 5.40. Found: C, 69.57; H, 9.86; N, 5.20.

5.3.15. (2*RS*,4*R*)-2-(2-Methoxy-phenyl)-thiazolidine-4-carboxylic acid hexadecylamide (3ca)—Yield: 38.8%. ¹H NMR (300 MHz, CDCl₃) δ 7.52–7.27 and 7.04–6.92 (m, 4H), 7.35 (br, 1H), 5.93 and 5.63 (s, s, 1H), 4.37 and 4.23 (dd, *J* = 7.8 Hz and 4.2 Hz, br, 1H), 3.92 and 3.89 (s, s, 3H), 3.67, 3.42–3.35 (dd, m, 2H), 3.33–3.20 (m, 2H),

1.53 (m, 2H), 1.25 (s, 26H), 0.88 (t, 3H, $J = 6.9$ Hz). MS (ESI) m/z 463.4 $[M+H]^+$. Anal. Calcd for $C_{27}H_{46}N_2O_2S$: C, 70.08; H, 10.02; N, 6.05. Found: C, 69.97; H, 9.96; N, 6.02.

5.3.16. (2*RS*,4*R*)-2-(3-Methoxy-phenyl)-thiazolidine-4-carboxylic acid

hexadecylamide (3da)—Yield: 33.6%. 1H NMR (300 MHz, $CDCl_3$) δ 7.47–6.87 (four doublets, 4H, $J = 8.7$ Hz), 7.30 (br, 1H), 5.64 and 5.33 (s, s, 1H), 4.36 and 4.25 (dd, $J = 4.2$ Hz, br, 1H), 3.88 and 3.83 (s, s, 3H), 3.69, 3.46–3.35 (dd, $J = 10.2$ Hz and 4.2 Hz, m, 2H), 3.30–3.24 (m, 2H), 1.53–1.48 (m, 2H), 1.25 (s, 26H), 0.88 (t, 3H, $J = 7.2$ Hz). MS (ESI) m/z 485.5 $[M+Na]^+$. Anal. Calcd for $C_{27}H_{46}N_2O_2S$: C, 70.08; H, 10.02; N, 6.05. Found: C, 69.88; H, 9.93; N, 5.97.

5.3.17. (2*RS*,4*R*)-2-(4-Methoxy-phenyl)-thiazolidine-4-carboxylic acid

hexadecylamide (3ea)—Yield: 28.8%. 1H NMR (300 MHz, $CDCl_3$) δ 7.86–6.88 (m, 4H), 7.36 (br, 1H), 5.69 and 5.32 (s, s, 1H), 4.39 and 4.46 (dd, br, 1H), 3.90 and 3.82 (s, s, 3H), 3.68, 3.50–3.40 (dd, m, 2H), 3.30–3.24 (m, 2H), 1.53–1.45 (m, 2H), 1.26 (s, 26H), 0.88 (t, 3H, $J = 6.9$ Hz). MS (ESI) m/z 463.1 $[M+H]^+$. Anal. Calcd for $C_{27}H_{46}N_2O_2S$: C, 70.08; H, 10.02; N, 6.05. Found: C, 70.13; H, 10.12; N, 6.00.

5.3.18. (2*RS*,4*R*)-2-(4-Dimethylamino-phenyl)-thiazolidine-4-carboxylic acid

hexadecylamide (3fa)—Yield: 62.5%. 1H NMR (300 MHz, $CDCl_3$) δ 7.33 and 6.70 (dd, $J = 8.7$ Hz, 4H), 7.39 and 6.43 (br, 1H), 5.55 and 5.25 (s, s, 1H), 4.33 and 3.87 (dd, $J = 4.2$ Hz, t, 1H), 3.69 and 3.45–3.39 (dd, $J = 4.2$ Hz, m, 2H), 3.32–3.12 (m, 2H), 2.96 and 2.95 (s, s, 3H), 1.55–1.46 (m, 2H), 1.25 (s, 26H), 0.87 (t, 3H). MS (ESI) m/z 476.4 $[M+H]^+$. Anal. Calcd for $C_{28}H_{49}N_3OS$: C, 70.68; H, 10.38; N, 8.83. Found: C, 70.61; H, 10.49; N, 8.70.

5.3.19. (2*RS*,4*R*)-2-(2-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid

hexadecylamide (3ga)—Yield: 52.7%. 1H NMR (300 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.87 (d, $J = 7.8$ Hz, 1H), 7.50 (d, $J = 7.8$ Hz, 1H), 7.35 (t, $J = 7.8$ Hz, 1H), 7.16 (t, $J = 7.8$ Hz, 1H), 6.78 (br, 1H), 5.52 and 5.48 (s, s, 1H), 4.16 (br, 1H), 3.61 and 3.39–3.32 (dd, m, 2H), 3.30–3.20 (m, 2H), 2.90 (br, 1H), 2.25 (s, 3H), 1.54–1.50 (m, 2H), 1.25 (s, 26H), 0.87 (t, 3H, $J = 6.9$ Hz). MS (ESI) m/z 512.3 $[M+Na]^+$, 488.1 $[M-h]^-$. Anal. Calcd for $C_{28}H_{47}N_3O_2S$: C, 68.51; H, 9.61; N, 8.48. Found: C, 68.57; H, 9.55; N, 8.47.

5.3.20. (2*RS*,4*R*)-2-(3-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid

hexadecylamide (3ha)—Yield: 70.1%. 1H NMR (300 MHz, $CDCl_3$) δ 7.75 and 7.65 (s, s, 0.7H, 0.3H), 7.51–7.22 (m, 4H), 7.19 and 6.48 (br, 1H), 5.59 and 5.34 (s, s, 0.3H, 0.7H), 4.32 and 3.93 (dd, $J = 3.9$ Hz, t, $J = 6.9$ Hz, 0.7H, 0.3H), 3.68 and 3.42–3.34 (dd, $J = 3.9$ Hz, m, 2H), 3.31–3.16 (m, 2H), 2.60 (br, 1H), 2.17 (s, 3H, $J = 6.9$ Hz), 1.52 (m, 2H), 1.25 (s, 26H), 0.87 (t, 3H, $J = 6.9$ Hz). MS (ESI) m/z 512.3 $[M+Na]^+$. Anal. Calcd for $C_{28}H_{47}N_3O_2S$: C, 68.51; H, 9.61; N, 8.48. Found: C, 68.54; H, 9.65; N, 8.49.

5.3.21. (2*RS*,4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid (1-

methyl-octadecyl)-amide (3ia)—Yield: 68.7%. 1H NMR (300 MHz, $CDCl_3$) δ 9.91 (s, 1H) 7.56 (s, br, 4H), 7.49 (s, br, 1H), 5.77 and 5.76 (s, s, 1H), 4.89 and 4.79 (dd, $J = 3.9$ Hz, t, br, 0.6H, 0.4H), 3.67 and 3.44 (m, t, 2H), 3.37–3.19 (m, 2H), 3.08 and 2.97 (s, s, 3H), 2.18 (s, 3H), 1.56 (m, 2H), 1.25 (s, 30H), 0.87 (t, 3H, $J = 7.2$ Hz). MS (ESI) m/z 532.3 $[M+Na]^+$.

Anal. Calcd for $C_{31}H_{53}N_3O_2SCF_3COOH \cdot H_2O$: C, 59.70; H, 8.50; N, 6.33. Found: C, 60.15; H, 8.71; N, 6.27.

5.3.22. (2*RS*,4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid adamantan-1-ylamide (3ib)—Yield: 73.0%. 1H NMR (300 MHz, DMSO- d_6) δ 10.04 (s, 1H), 7.79 (br, 1H), 7.50 (dd, J = 8.7 Hz, 4H), 5.74 and 5.58 (s, s, 1H), 4.27 and 3.96 (br, 1H), 3.45–3.35 and 3.16–2.99 (m, m, 2H), 2.04 (s, 3H), 1.94 (s, 6H), 1.62 (s, 6H). MS (ESI) m/z 400.3 $[M+H]^+$. Anal. Calcd for $C_{22}H_{29}N_3O_2SCF_3COOH$: C, 56.13; H, 5.89; N, 8.18. Found: C, 56.39; H, 5.89; N, 8.18.

5.3.23. (2*RS*,4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid adamantan-2-ylamide (3ic)—Yield: 45.3%. 1H NMR (300 MHz, DMSO- d_6) δ 9.99 (s, 0.5H), 8.08 and 8.02 (dd, J = 7.2 Hz, 1H), 7.58–7.38 (m, 4H), 5.56 and 5.53 (s, s, 1H), 4.30 and 3.88 (t, J = 6.0 Hz, br, 1H), 3.36 and 3.16–2.93 (dd, m, 2H), 2.03 (s, 3H), 1.82–1.49 (m, 15H). MS (ESI) m/z 400.3 $[M+H]^+$, 398.0 $[M-H]^-$. Anal. Calcd for $C_{22}H_{29}N_3O_2S1/2 CF_3COOH$: C, 60.51; H, 6.51; N, 9.20. Found: C, 60.62; H, 6.83; N, 8.93.

5.3.24. (2*RS*,4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid-(9*H*-fluoren-2-yl)-amide (3id)—Yield: 45.7%. 1H NMR (300 MHz, DMSO- d_6) δ 10.33 and 10.13 (s, s, 1H), 10.00 and 9.97 (s, s, 1H), 7.97 and 7.94 (s, br, 1H), 7.84–7.24 (m, 7H), 7.63–7.24 (m, 4H), 5.69 and 5.59 (s, s, 1H), 4.39 and 4.04 (t, J = 5.7 Hz, m, 1H), 3.91 (s, 2H), 3.47–3.29 and 3.13 (m, 2H), 2.05 (s, 3H). MS (ESI) m/z 430.1 $[M+H]^+$. Anal. Calcd for $C_{25}H_{23}N_3O_2S CF_3- COOH$: C, 56.13; H, 5.89; N, 8.18. Found: C, 56.01; H, 5.87; N, 8.01.

5.3.25. (2*RS*,4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid anthracen-2-yl)-amide (3ie)—Yield: 45.7%. 1H NMR (300 MHz, DMSO- d_6) δ 10.49 and 10.27 (s, s, 1H), 10.00 and 9.97 (s, s, 1H), 8.55–8.46 (m, 3H), 8.06–8.03 (m, 3H), 7.68–7.44 (m, 4H), 7.58–7.44 (m, 4H), 5.70 and 5.60 (s, s, 1H), 4.43 and 4.07 (t, br, 1H), 3.49–3.35 and 3.17 (m, t, 2H), 2.04 (s, 3H). MS (ESI) m/z 442.1 $[M+H]^+$. Anal. Calcd for $C_{26}H_{23}N_3O_2SCF_3COOH$: C, 60.53; H, 4.35; N, 7.56. Found: C, 60.31; H, 4.37; N, 7.71.

5.3.26. (2*RS*,4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid biphenyl-4-ylamide (3if)—Yield: 35.1%. 1H NMR (300 MHz, $CDCl_3$) δ 9.27 and 8.74 (s, s, 1H), 7.83–7.30 (m, 13H), 7.17 and 6.90 (s, br, 1H), 5.68 and 5.40 (s, 0.3H and s, 0.7H), 4.53 and 4.21 (dd, J = 3.9 Hz, t, 1H), 3.84–3.79 and 3.59–3.43 (dd, , J = 3.9 Hz, m, 2H), 2.16 and 2.19 (s, s, 3H). MS (ESI) m/z 440.1 $[M+Na]^+$, 416.0 $[M-H]^-$. Anal. Calcd for $C_{24}H_{23}N_3O_2S$: C, 69.04; H, 5.55; N, 10.06. Found: C, 68.87; H, 5.32; N, 10.01.

5.3.27. (2*RS*,4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid benzothiazol-2-ylamide (3ig)—Yield: 37.9%. 1H NMR (300 MHz, $CDCl_3$) δ 10.56 (br, 1H), 7.857.78 (dd, J = 14.4 Hz and 7.8 Hz, 2H), 7.55–7.41 (m, 5H), 7.36–7.29 (m, 2H), 5.68 and 5.41 (s, 0.15H and d, J = 9.6 Hz, 0.85H), 4.64 and 4.32 (br, t, 1H), 3.80 and 3.60–3.44 (m, 2H), 2.79 (br, 1H), 2.19 (s, 3H). MS (ESI) m/z 421.1 $[M+Na]^+$. Anal. Calcd for $C_{19}H_{18}N_4O_2S_2$: C, 57.26; H, 4.55; N, 14.06. Found: C, 57.17; H, 4.32; N, 14.01.

5.3.28. (2*RS*,4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid hexadec-9-*cis*-enylamide (3ih)—Yield: 18.1%. ¹H NMR (300 MHz, CDCl₃) δ 7.86–7.41 (m, 4H), 7.30 (br, 1H), 7.09 and 6.42 (br, t, 1H), 5.57 and 5.39 (s, s, 1h), 5.37–5.32 (m, 2H), 4.32 and 3.89 (dd, *J* = 4.2 Hz, t, *J* = 7.8 Hz, 1h), 3.68 and 3.43–3.36 (dd, *J* = 4.2 Hz, m, 2H), 3.31–3.20 (m, 2H), 2.51 (br, 1H), 2.18 (s, 3H), 2.01–1.97 (br, 4H), 1.54–1.46 (m, 2H), 1.29 and 1.27 (s, s, 18H), 0.87 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 510.4 [M + Na]⁺, 486.1 [M – H][–]. Anal. Calcd for C₂₅H₄₈N₃O₂S: C, 68.95; H, 9.30; N, 8.62.

5.3.29. (2*RS*,4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid nonadec-10-ynylamide (3ii)—Yield: 40.5%. ¹H NMR (300 MHz, CDCl₃) δ 7.83–7.41 (m, 4H), 7.26 (br, 1H), 7.23 and 6.43 (br, br, 1H), 5.57 and 5.31 (s, s, 1h), 4.32 and 3.90 (dd, *J* = 4.2 Hz, t, *J* = 7.2 Hz, 1H), 3.68 and 3.43–3.36 (dd, *J* = 4.2 Hz, m, 2H), 3.32–3.20 (m, 2H), 2.51 (br, 1H), 2.18 (s, 3H), 2.13 (t, 4H), 1.54–1.44 (m, 2H), 1.31 and 1.27 (s, s, 22H), 0.87 (t, 3H, *J* = 6.6 Hz). MS (ESI) *m/z* 514.3 [M + H]⁺, 512.1 [M – H][–]. Anal. Calcd for C₃₀H₄₇N₃O₂S: C, 70.13; H, 9.22; N, 8.18. Found: C, 70.21; H, 9.14; N, 8.05.

5.3.30. (2*RS*,4*R*)-2-Phenyl-thiazolidine-4-carboxylic acid hexadecylamide (3ja)—Yield: 39.3%. ¹H NMR (300 MHz, CDCl₃) δ 7.55–7.31 (m, 5H), 7.26 and 6.41 (br, 1H), 5.62 and 5.34 (d, *J* = 9.9 Hz, d, *J* = 11.4 Hz, 1H), 4.35 and 3.93 (dt, dd, *J* = 7.5 Hz and 4.2 Hz, 1H), 3.71 and 3.45–3.30 (dd, *J* = 4.2 Hz m, 2H), 3.30–3.19 (m, 2H), 2.66 and 2.53 (br, 1H), 1.52 (m, 2H), 1.25 (s, 26H), 0.88 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 455.4 [M + Na]⁺, 431.1 [M – H][–]. Anal. Calcd for C₂₆H₄₄N₂OS: C, 72.17; H, 10.25; N, 6.47. Found: C, 71.98; H, 10.02; N, 6.34.

5.3.31. (2*RS*,4*R*)-2-Phenyl-thiazolidine-4-carboxylic acid (*Z*)-octadec-8-enylamide (3jb)—Yield: 46.1%. ¹H NMR (300 MHz, CDCl₃) δ 7.53–7.31 (m, 5H), 7.26 and 6.42 (br, br, 1H), 5.61 and 5.34 (s, br, 1H), 5.34 (br, 2H), 4.35 and 3.93 (dd, *J* = 4.2 Hz, t, *J* = 7.5 Hz, 0.7H and 0.3H), 3.71 and 3.45–3.39 (dd, *J* = 4.2 Hz, m, 2H), 3.37–3.19 (m, 2H), 2.54 (br, 1H), 2.01–1.99 (m, 4H), 1.52–1.45 (m, 2H), 1.29 and 1.26 (s, 22H), 0.87 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 459.4 [M + H]⁺. Anal. Calcd for C₂₈H₄₆N₂OS: C, 73.31; H, 10.11; N, 6.11. Found: C, 73.15; H, 10.09; N, 6.14.

5.3.32. (2*RS*,4*R*)-2-Phenyl-thiazolidine-4-carboxylic acid (*E*)-octadec-8-enylamide (3jc)—Yield: 35.7%. ¹H NMR (300 MHz, CDCl₃) δ 7.47–7.31 (m, 5H), 7.26 and 6.42 (br, br, 1H), 5.61 and 5.37 (br, br, 1H), 5.34–5.33 (br, *J* = 13.8 Hz, 2H), 4.35 and 3.91 (br, br, 0.7H and 0.3H), 3.71 and 3.45–3.37 (dd, *J* = 4.2 Hz, m, 2H), 3.34–3.19 (m, 2H), 2.66 and 2.54 (br, 1H), 1.96–1.95 (m, 4H), 1.54–1.42 (m, 2H), 1.26 (s, 22H), 0.87 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 459.4 [M + H]⁺, 457.1 [M – H][–]. Anal. Calcd for C₂₈H₄₆N₂OS: C, 73.31; H, 10.11; N, 6.11. Found: C, 73.36; H, 10.16; N, 6.12.

5.4. (2*RS*,4*R*)-3-Acetyl-2-(4-acetylamino-phenyl)-thiazolidine-4-carboxylic acid hexadecylamide (4)

Under 0 °C, to a mixture of **1b** (0.30 g, 0.61 mmol) and pyridine (0.10 g, 1.26 mmol) in 10 mL CH₂Cl₂, was added acetyl chloride (0.19 g, 2.42 mmol) and stirred at room temperature for 8 h. The reaction mixture was washed with satd NaHCO₃ and dried over MgSO₄. The

solvent was concentrated and purified through column chromatography to yield **4** (0.24 g, 73.6%) as a white solid. ^1H NMR (300 MHz, CDCl_3) δ 7.50 (br, 1H), 7.50–7.34 (d, J = 8.4 Hz, 4H), 7.02 and 6.55 (br, br, 1H), 5.97 (s, 1H), 5.07 and 4.70 (br, br, 1H), 3.67 and 3.14–3.08 (dd, J = 4.2 Hz, br, 2H), 3.31–3.29 (m, 2H), 2.17 (s, 3H), 1.98 (s, 3H), 1.54 (m, 2H), 1.25 (s, 26H), 0.87 (t, 3H, J = 6.9 Hz). MS (ESI) m/z 554.5 $[\text{M}+\text{Na}]^+$, 530.2 $[\text{M}-\text{H}]^-$. Anal. Calcd for $\text{C}_{30}\text{H}_{49}\text{N}_3\text{O}_3\text{S}$: C, 67.76; H, 9.29; N, 7.90. Found: C, 67.49; H, 9.40; N, 7.75.

5.5. (2*RS*,4*R*)-2-(4-Acetylamino-phenyl)-thiazolidine-4-carboxylic acid hexadecyl ester (**5**)

A mixture of Boc-protected carboxylic acid **2i** (0.549 g, 1.50 mmol), DCC (0.372 g, 1.8 mmol), DMAP (0.055 g, 0.45 mmol), (1*S*)-(+)-10-camphorsulfonic acid (0.105 g, 0.45 mmol), and hexadecanol (0.726 g, 3.0 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature for 24 h. The reaction mixture was filtered, then washed with satd NaHCO_3 and brine (10 mL), and dried over MgSO_4 . The pure Boc-protected ester was obtained after column chromatography (0.83 g, 93.8%). To a solution of Boc-protected ester (0.83 g, 1.41 mmol) in CH_2Cl_2 (10 mL) was added TFA (1 mL) and stirred for two days. The reaction mixture was concentrated, washed with satd NaHCO_3 , and dried over MgSO_4 . The solvent was removed, and the crude compound was purified by column chromatography using hexane/ethyl acetate gradient elution system to yield **5** as a white solid (0.41 g, 59.5%). ^1H NMR (CDCl_3) δ 7.52–7.45 (m, 4H), 7.21 and 7.17 (br, br, 1H), 5.79 and 5.52 (s, 1H), 4.19 (t, 2H, J = 6.6 Hz), 4.18–4.14 and 3.96 (m, t, 1H), 3.493.35 and 3.20–3.07 (m, m, 2H), 2.63 (br, 1H), 2.18 and 2.17 (s, s, 3H), 1.69–1.62 (m, 2H), 1.25 (s, 26H), 0.88 (t, 3H); MS (ESI) m/z 513.3 $[\text{M}+\text{Na}]^+$, 489.1 $[\text{M}-\text{H}]^-$. Anal. Calcd for $\text{C}_{28}\text{H}_{46}\text{N}_2\text{O}_3\text{S}$: C, 68.53; H, 9.45; N, 5.71. Found: C, 65.47; H, 9.40; N, 5.75.

5.6. General procedure for preparing (2*RS*,4*R*)-alkyl-2-aryl-thiazolidin-4-ylmethyl)-amine **8a-8b**

To a mixture of Boc-protected carboxylic acid **2j** (5.6 mmol), EDCI (6.7 mmol), HOBT (5.6 mmol), and NMM (13.4 mmol) in CH_2Cl_2 (20 mL) was added *O,N*-dimethyl-hydroxylamine hydrochloride (5.9 mmol); the mixture was stirred continuously at room temperature for 10 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and sequentially washed with water, 5% HCl, satd NaHCO_3 , and brine and dried over MgSO_4 . The solvent was removed under reduced pressure to yield a crude product methoxy-methylamide **6**, which was recrystallized from ethyl ether/hexane as white crystals and used directly in the next step without purification (71%). Under -40°C , LAH (1.0 mL, 2 M in THF) was added to a solution of 4-(methoxy-methyl-carbamoyl)-2-phenyl-thiazolidine-3-carboxylic acid *tert*-butyl ester (0.20 g, 0.57 mmol) in THF (10 mL) and stirred for 30 min. The reaction mixture was quenched with ethyl acetate (10 mL) and satd NH_4Cl (10 mL). Then 5% H_2SO_4 (20 mL) was added under 0°C . The mixture was extracted with ethyl acetate and dried with MgSO_4 . The solvent was removed and pure aldehyde **7** (0.14 g, 84.3%) was purified as a colorless oil after column chromatography. To a solution of **7** (0.14 g) in MeOH (3 mL) was charged hexadecylamine (1 equiv) in MeOH (7 mL). After stirring for 10 min, HOAc (1.3 equiv) and NaBH_3CN (2.5 equiv) were added and stirred for 2 h. The reaction mixture was quenched with satd NaHCO_3 (10 mL), extracted with ethyl acetate, and dried with MgSO_4 . The solvent was removed under reduced pressure to yield a crude 4-

hexadecylaminomethyl-2-phenyl-thiazolidine-3-carboxylic acid *tert*-butyl ester. Deprotection with TFA yielded product **8a**. Then **8a** was converted to a corresponding hydrochloride by using 2 M HCl/Et₂O. Compound **8b** was prepared using the same method.

5.6.1. (2*RS*,4*R*)-Hexadecyl-(2-phenyl-thiazolidin-4-ylmethyl)-amine

hydrochloride (8a)—Yield: 42.9%. ¹H NMR (300 Hz, CDCl₃) δ 7.66–7.38 (m, 5H), 5.87 (s, 0.55H), 5.79 (s, 0.45H), 4.34 and 3.96 (br, 1H), 3.34–3.32 (m, 2H), 3.31–3.08 (m, 2H), 2.92 (m, 2H), 1.64 (m, 2H), 1.24 (s, 26H), 0.85 (t, 3H, *J*=6.6 Hz). MS (ESI) *m/z* 419.1 [M + H]⁺. Anal. Calcd for C₂₆H₄₆N₂S ■ 2HCl ■ 1/2H₂O: C, 62.37; H, 9.86; N, 5.60. Found: C, 62.29; H, 9.91; N, 5.80.

5.6.2. (2*RS*,4*R*)-Hexadecyl-(2-(4-acetyl-phenyl-thiazolidin-4-ylmethyl)-amine

hydrochloride (8b)—Yield: 48.4%. ¹H NMR (300 Hz, CDCl₃) δ 7.35–7.57 (m, 4H), 5.52 (s, 0.55H), 5.47 (s, 0.45H), 4.02, 3.52 (br, 1H), 3.00–3.24 (m, 2H), 2.61–2.92 (m, 4H), 2.17 (s, 3H), 1.48–1.61 (m, 2H), 1.25 (s, 26H), 0.87 (t, 3H, *J*= 6.9 Hz); MS (ESI) *m/z* 476.4 [M + H]⁺. Anal. Calcd for C₂₈H₄₉N₃OS HCl: C, 65.65; H, 9.84; N, 8.20. Found: C, 65.55; H, 9.73; N, 8.04.

5.7. Synthesis of disulfanyl dimer (9)

Under 0 °C, to a solution of **1b** (330 mg, 0.67 mmol) in THF (10 mL) was charged B₂H₆ (8.0 mL, 1 M in THF) and refluxed for 24 h. Then the reaction mixture was quenched by satd NH₄Cl and dried over MgSO₄. Disulfanyl dimer **9b** was separated from a silica gel column using hexane/ethyl acetate gradient elution system (127 mg, 41%). ¹H NMR (500 MHz, CDCl₃) δ 7.44 and 7.21 (d, d, 8H), 7.40 (t, 2H, *J*= 6.0 Hz), 3.69 (q, 4H), 3.37 (q, 2H, *J*= 4.0 Hz), 3.29–3.18 (m, 4H), 3.13 (dd, 2H, *J*= 4.0 Hz, *J*=14.0 Hz), 2.71 (dd, 2H, *J*=14.0 Hz, *J*= 8.5 Hz), 2.17 (s, 6H), 1.51–1.48 (m, 4H), 1.25 and 1.29 (s, 52H), 0.87 (t, 6H). MS (ESI) *m/z* 1003.9 [M+Na]⁺. Anal. Calcd for C₅₆H₉₆N₆O₄S₂: C, 68.53; H, 9.86; N, 8.56. Found: C, 68.54; H, 9.74; N, 8.31.

5.8. Synthesis of (2*RS*,4*R*)-2-(4-acetyl-amino-phenyl)-thiazolidine-3,4-dicarboxylic acid 3-(9*H*-fluoren-9-ylmethyl) ester (10)

At 0 °C, to a suspension of (2*RS*,4*R*)-2-(4-acetylaminophenyl)-thiazolidine-4-carboxylic acid (1.00 g, 3.76 mmol) in 15 mL CH₂Cl₂ was added triethylamine (0.38 g, 3.80 mmol) and 9-fluorenylmethyl chloroformate (0.98 g, 3.80 mmol). After stirring for 30 min, the mixture was washed with 5% HCl and dried with MgSO₄. The solvent was removed, and crude compound was purified by column chromatography using hexane/ethyl acetate gradient elution system to yield Fmoc-protected acid as a white foam **10** (1.82 g, 99%). Yield: 99%. ¹H NMR (300 MHz, CDCl₃) δ 10.24 (br, 1H), 7.73–7.05 (m, 13H), 6.14–5.85 (m, 1H), 5.05–4.38 (m, 3H), 3.99 (br, 1H), 3.36–3.23 (m, 2H), 2.22 (s, 3H). MS (ESI) *m/z* 511.1 [M + Na]⁺, 486.9 [M – H][–].

5.9. Preparation of (2*RS*,4*R*)-2-(4-aminophenyl)-thiazolidine-4-carboxylic acid amide **13a-13b**

The Fmoc-protected acid **10** (3.97 g, 8.135 mmol), EDCI (1.2 equiv) and HOBT (1.0 equiv) in CH₂Cl₂ (80 mL) was stirred at room temperature for 10 min. To this solution,

dodecylamine or hexadecylamine (1.0 equiv) and Et₃N (1.2 equiv) were added and stirred continuously at room temperature for 3.5 h. The reaction mixture was sequentially washed with water, satd NaHCO₃, and brine, and then dried over MgSO₄. The solvent was removed under reduced pressure to yield a crude oil **11** (4.11 g, 70.98%), which was used in the next step without purification. **11** (2.0 g) was added to a solution of CH₃COCl (4.5 mL) in MeOH (10 mL) under 0 °C and stirred at room temperature for four days. The solvent was evaporated on vacuum and diluted with CH₂Cl₂ (30 mL), then washed with satd NaHCO₃, and dried over MgSO₄ to obtain the crude compound **12** as a yellow solid (0.9 g, 47.8%). To a solution of **12** (1 equiv) in CH₂Cl₂ (10 mL) was added 1,8-diazabicycloundec-7-ene (1.5 equiv) and stirred for 30 min to 1 h. The mixture was washed with 2.5% HCl, extracted with CH₂Cl₂, and dried with MgSO₄. The solvent was removed on vacuum and purified by column chromatography using hexane/ethyl acetate gradient elution system to give **13a** and **13b** as a light yellow solid.

5.9.1. (2*RS*,4*R*)-2-(4-Amino-phenyl)-thiazolidine-4-carboxylic acid

dodecylamide (13a)—Yield: 65.7%. ¹H NMR (300 MHz, CDCl₃) δ 7.31–7.24 (m, 4H), 7.29 and 6.39 (br, br, 1H), 5.51 and 5.24 (br, 0.3H, d, *J* = 9.6 Hz, 0.7H), 4.32 and 3.86 (br, br, 1H), 3.74 (br, 2H), 3.67 and 3.40 (dd, dd, *J* = 4.2 Hz, *J* = 8.1 Hz, 2H), 3.29–3.20 (m, 2H), 2.44 (br, 1H), 1.53–1.47 (m, 2H), 1.25 (s, 18H), 0.87 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 392.4 [M+H]⁺, 390.1 [M–H][–]. Anal. Calcd for C₂₂H₃₇N₃OS: C, 67.47; H, 9.52; N, 10.73. Found: C, 67.34; H, 9.52; N, 10.65.

5.9.2. (2*RS*,4*R*)-2-(4-Amino-phenyl)-thiazolidine-4-carboxylic acid

hexadecylamide (13b)—Yield: 53.2%. ¹H NMR (300 MHz, CDCl₃) δ 7.31–7.25 (m, 4H), 7.26 and 6.39 (br, br, 1H), 5.52 and 5.23 (br, 0.3H, br, 0.7H), 4.32 and 3.86 (br, m, 1H), 3.72 (br, 2H), 3.68 and 3.41 (dd, *J* = 3.9 Hz, dd, *J* = 8.1 Hz, 2H), 3.29–3.20 (m, 2H), 2.44 (br, 1H), 1.51–1.47 (m, 2H), 1.25 (s, 26H), 0.88 (t, 3H, *J* = 6.9 Hz). MS (ESI) *m/z* 448.5 [M+H]⁺, 446.1 [M–H][–]. Anal. Calcd for C₂₆H₄₅N₃OS: C, 69.75; H, 10.13; N, 9.39. Found: C, 69.80; H, 10.21; N, 9.16.

5.10. General procedure for preparing (2*RS*,4*R*)-2-(4-amido-phenyl)-thiazolidine-4-carboxylic acid hexadecylamide **15a–15c**

5.10.1. (2*RS*,4*R*)-2-(4-Methanesulfonylaminophenyl)-thiazolidine-4-carboxylic acid hexadecylamide (15a)—At 0 °C, to a solution of **12** (450 mg, 0.672 mmol) in CH₂Cl₂ (10 mL) were added pyridine (88 mg, 1.11 mmol) and methanesulfonyl chloride (144 mg, 1.25 mmol) and stirred at room temperature for 10 h. The reaction mixture was washed with satd NaHCO₃ and dried over MgSO₄. The solvent was removed to yield the crude Fmoc-protected product **14a**, which was used in the next step without further purification (79.7%). To the solution of **14a** (310 mg, 0.414 mmol) in CH₂Cl₂ (10 mL) was added 1, 8-diazabicycloundec-7-ene (94.5 mg, 0.622 mmol) and stirred for 1 h. The mixture was washed with 2.5% HCl, extracted with CH₂Cl₂, and dried with MgSO₄. The solvent was removed on vacuum and purified by column chromatography using hexane/ethyl acetate gradient elution system to give **15a** as a white solid. Yield: 45.9%. ¹H NMR (300 MHz, CDCl₃) δ 7.52–7.46 and 7.24–7.19 (m, 4H), 7.15 and 6.33 (br, 0.3H, br, 0.7H), 6.52 (br, 1H), 5.57 and 5.33 (d, *J* = 10.8 Hz, 0.3H, d, *J* = 11.4 Hz, 0.7H), 4.33 and 3.89 (br, br, 0.7H)

and 0.3H), 3.69 and 3.42 (dd, $J = 4.2$ Hz, m, 2H), 3.32–3.22 (m, 2H), 3.02 and 3.01 (s, s, 3H), 2.65 and 2.50 (br, 1H), 1.52–1.48 (m, 2H), 1.25 (s, 26H), 0.87 (t, 3H, $J = 6.6$ Hz). MS (ESI) m/z 548.4 $[M+Na]^+$, 524.2 $[M-H]^-$. Anal. Calcd for $C_{27}H_{47}N_3O_3S_2$: C, 61.67; H, 9.01; N, 7.99. Found: C, 61.91; H, 9.03; N, 7.91.

5.10.2. (2*RS*,4*R*)-2-[4-(2-Chloro-acetylamino)-phenyl]-thiazolidine-4-carboxylic acid hexadecylamide (15b)—At 0 °C, to a solution of **12** (390 mg, 0.582 mmol) in CH_2Cl_2 (10 mL) were added pyridine (72 mg, 9.11 mmol) and chloro-acetyl chloride (122 mg, 1.08 mmol) and stirred at room temperature for 10 h. The reaction mixture was washed with satd $NaHCO_3$ and dried over $MgSO_4$. The solvents were removed to yield the crude Fmoc-protected product **14b**, which was used in the next step without further purification (50.7%). To the solution of **14b** (220 mg, 0.295 mmol) in CH_2Cl_2 (10 mL) was added 1, 8-diazabicyclo[4.3.0]undec-7-ene (103 mg, 0.678 mmol) and stirred for 20 min. The mixture was washed with 1 N HCl, extracted with CH_2Cl_2 , and dried with $MgSO_4$. The solvent was removed on vacuum and the resulting crude material was purified by column chromatography using hexane/ethyl acetate gradient elution system to give **15b** as a white solid. Yield: 53.2%. 1H NMR (300 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.58–7.46 (m, 4H), 7.19 and 6.38 (br, br, 1H), 5.59 and 5.33 (s, s, 1H), 4.34 and 3.91 (br, br, 0.7H and 0.3H), 4.20 (s, 2H), 3.69 and 3.39 (dd, $J = 3.9$ Hz, br, 2H), 3.33–3.26 (m, 2H), 2.52 (br, 1H), 1.56 (br, 2H), 1.25 (s, 26H), 0.87 (t, 3H, $J = 6.0$ Hz). MS (ESI) m/z 541.5 $[M+Na]^+$, 522.3 $[M-H]^-$. Anal. Calcd for $C_{28}H_{46}ClN_3O_3S$: C, 64.15; H, 8.84; N, 8.02. Found: C, 64.06; H, 8.88; N, 7.91.

5.10.3. (2*RS*,4*R*)-2-(4-Ureido-phenyl)-thiazolidine-4-carboxylic acid hexadecylamide (15c)—At 0 °C, to a solution of **12** (240 mg, 0.32 mmol) in water (5 mL) were added sodium cyanate (64 mg, 0.98 mmol) and acetic acid (0.15 mL). The mixture was stirred at 50 °C for 24 h. It was extracted with ethyl acetate and dried over $MgSO_4$. The solvents were removed to yield the crude Fmoc-protected product **14c**, which was used in the next step without further purification (87.7%). To the solution of **14c** (200 mg, 0.28 mmol) in CH_2Cl_2 (10 mL) was added 1,8-diazabicyclo[4.3.0]undec-7-ene (99 mg, 0.65 mmol) and stirred for 45 min. The mixture was washed with 1 N HCl, extracted with CH_2Cl_2 , and dried with $MgSO_4$. The solvent was removed on vacuum, and the resulting crude material was purified by column chromatography using hexane/ethyl acetate gradient elution system to give **15c**. Yield: 51.1%. 1H NMR (300 MHz, $CDCl_3$) δ 7.56–7.33 (m, 4H), 7.28 and 6.46 (br, 1H), 6.82 (br, 1H), 5.57 and 5.33 (s, 0.3H, s, 0.7H), 4.78 (s, 2H), 4.33 and 3.91 (dd, $J = 4.2$ Hz, t, $J = 7.5$ Hz, 0.7H and 0.3H), 3.68 and 3.41 (dd, $J = 11.1$ Hz and 4.2, m, 2H), 3.32–3.25 (m, 2H), 2.60 (br, 1H), 1.56–1.45 (m, 2H), 1.27 (s, 26H), 0.89 (t, 3H, $J = 6.9$ Hz). MS (ESI) m/z 513.5 $[M+Na]^+$, 491.4 $[M+H]^+$, 489.1 $[M-H]^-$. Anal. Calcd for $C_{27}H_{48}N_4O_3S_2 \cdot H_2O$: C, 63.74; H, 9.51; N, 11.01. Found: C, 63.89; H, 9.01; N, 10.91.

5.11. (2*R*)-2-Amino-*N*-hexadecyl-3-methylsulfanyl-propion-amide (16a)

At 0 °C, (2*R*)-2-amino-3-methylsulfanyl-propionic acid (2.5 g, 18.5 mmol) was dissolved in 1 N NaOH (20 mL) and 1,4-dioxane (40 mL); then di-*tert*-butyldicarbonate (8.1 g, 37 mmol) was added slowly and stirred at room temperature overnight. The reaction mixture was concentrated in vacuum and washed with ethyl acetate (20 mL). The aqueous phase was adjusted to a pH 3 by adding 1 N HCl, then extracted with ethyl acetate, dried with

magnesium sulfate, filtered, and concentrated on vacuum to give Boc-protected (2*R*)-2-amino-3-methylsulfanyl-propionic acid as a white solid (90.96%). A mixture of Boc-protected (2*R*)-2-amino-3-methylsulfanyl-propionic acid (1.76 g, 7.5 mmol), EDCI (1.2 equiv), and HOBT (1.0 equiv) in CH₂Cl₂ (35 mL) was stirred at room temperature for 10 min. To this solution, hexadecylamine (1.0 equiv) and Et₃N (1.2 equiv) were added and stirring continuously at room temperature for 10 h. The reaction mixture was diluted with CH₂Cl₂ (50 mL) and sequentially washed with water, satd NaHCO₃, and brine, and dried over MgSO₄. The solvent was removed under reduced pressure to yield a crude oil, which was stirred with TFA (3 mL) in 20 mL CH₂Cl₂ at room temperature for 1 h to cleave the Boc group. The reaction mixture was concentrated, diluted with ethyl acetate, washed with satd NaHCO₃, and dried over MgSO₄. The solvent was removed to yield a crude solid, which was purified by column chromatography using hexane/ethyl acetate gradient elution system as a white solid. Yield: 63.4%. Mp (hexane) 55–56 °C. ¹H NMR (300 MHz, CDCl₃) δ 7.42 (br, 1H), 3.95 and 3.50 (br, dd, 1H, *J* = 3.9 Hz, 9.0 Hz), 3.24 (q, 2H), 3.02 and 2.66 (dd, dd, 2H, *J* = 3.9 Hz, *J* = 9.0 Hz, *J* = 13.8 Hz), 2.10 (s, 3H), 1.52–1.48 (m, 2H), 1.25 (s, 26H), 0.88 (t, 3H). MS (ESI) *m/z* 359.9 [M+H]⁺, 357.1 [M–H][–].

5.12. (2*R*)-2-(4-Acetyl-amino-benzyl-amino)-*N*-hexadecyl-3-methylsulfanyl-propionamide (16b)

To a suspension of **16a** (244 mg, 0.728 mmol) in MeOH (10 mL) was added *N*-(4-formylphenyl)-acetamide (119 mg, 0.728 mmol). After stirring for 5 min, HOAc (75 mg) was charged to the mixture and stirred for 20 min. Then NaBH₃CN (115 mg, 1.82 mmol) was added and stirred for 2 h. The reaction mixture was quenched with satd NaHCO₃, extracted with ethyl acetate, and dried on MgSO₄. The solvent was removed to yield a crude solid, which was purified by column chromatography using hexane/ethyl acetate gradient elution system. Yield: 61.1%. Mp (hexane) 83–84 °C. ¹H NMR (300 MHz, CDCl₃) δ 7.48–7.24 (m, 4H), 7.40 (br, 1H), 7.19 (br, 1H), 3.70 (q, *J* = 9.0 Hz, 2H), 3.28–3.18 (m, br, 3H), 2.98 (dd, *J* = 3.6 Hz, 1H), 2.58 (dd, *J* = 9.6 Hz, *J* = 13.8 Hz, 1H), 1.49–1.47 (m, 2H), 1.25 (s, 26H), 0.88 (t, 3H, *J* = 6.3 Hz). MS (ESI) *m/z* 506.4 [M+H]⁺, 504.1 [M–H][–]. Anal. Calcd for C₂₉H₅₁N₃O₂S: C, 68.86; H, 10.16; N, 8.31 Found: C, 69.98; H, 10.12; N, 8.47.

5.13. (2*R*)-2-Amino-3-benzylsulfanyl-*N*-hexadecyl-propionamide (16c)

A mixture of (2*R*)-3-benzylsulfanyl-2-*tert*-butoxycarbonyl-amino-propionic acid (0.5 g, 1.61 mmol), EDCI (0.371 g 1.93 mmol), and HOBT (0.228 g, 1.69 mmol) in CH₂Cl₂ (20 mL) was stirred at room temperature for 10 min. To this solution, hexadecylamine (0.409 g, 1.69 mmol) and Et₃N (0.195 g, 1.93 mmol) were added and stirred continuously at room temperature for 10 h. The reaction mixture was sequentially washed with water, satd NaHCO₃, and brine, and then dried over MgSO₄. The solvent was removed under reduced pressure to yield a crude product, which was stirred with TFA (3 mL) in 20 mL CH₂Cl₂ at room temperature for 2–3 h to cleave the Boc group. The reaction mixture was concentrated, diluted with ethyl acetate, washed with satd NaHCO₃, and dried over MgSO₄. The solvent was removed to yield a crude solid, which was purified by column chromatography using hexane/ethyl acetate gradient elution system as a white solid. Yield: 53.7%. Mp (hexane) 66–67 °C. ¹H NMR (300 MHz, CDCl₃ + D₂O) δ 7.51 (br, 1H), 7.35–7.20 (m, 5H), 3.75 (s, 2H), 3.70 (br, 1H), 3.28–3.13 (m, 2H), 2.99 (br, 1H), 2.90–2.68 (m, 1H), 1.48–1.46 (m, 2H),

1.25 (s, 26H), 0.88 (t, 3H, $J=6.9$ Hz). MS (ESI) m/z 435.4 $[M+H]^+$, 433.1 $[M-H]^-$. Anal. Calcd for $C_{26}H_{46}N_2O_2S$: C, 71.83; H, 10.67; N, 6.44. Found: C, 71.82; H, 10.51; N, 6.51.

5.14. 2-(4-Acetylamino-benzylamino)-*N*-hexadecyl-acetamide (16d)

At 0 °C, aminoacetic acid (2.08 g, 27.7 mmol) was dissolved in 1 N NaOH (28 mL) and 1,4-dioxane (25 mL); then di-*tert*-butyldi-carbonate (12.1 g, 55.4 mmol) was added slowly and stirred at room temperature overnight. The reaction mixture was concentrated in vacuum and washed with ethyl acetate (20 mL). The aqueous phase was adjusted to a pH 3 by adding 1 N HCl, then extracted with ethyl acetate, dried with magnesium sulfate, filtered, and concentrated on vacuum to give Boc-protected aminoacetic acid as a white crystalline solid (92.3%). A mixture of Boc-protected aminoacetic acid (0.87 g, 5 mmol), EDCI (1.152 g, 6 mmol), and HOBT (0.675 g, 5 mmol) in CH_2Cl_2 (25 mL) was stirred at room temperature for 10 min. To this solution, hexadecylamine (1.21 g, 5 mmol) and Et_3N (0.606 g, 6 mmol) were added and stirred continuously at room temperature for 10 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and sequentially washed with water, satd $NaHCO_3$, and brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a crude oil, which was stirred with TFA (3 mL) in 20 mL CH_2Cl_2 at room temperature for 1 h to cleave the Boc group. The reaction mixture was concentrated, diluted with EtOAc (50 mL), and washed with satd $NaHCO_3$. The white precipitate 2-amino-*N*-hexadecylacetamide was obtained by treatment with 1 N HCl (10 mL) (77.7%). To a suspension of 2-amino-*N*-hexadecylacetamide (200 mg, 0.60 mmol) in MeOH (15 mL) was added *N*-(4-formylphenyl)-acetamide (100 mg, 0.61 mmol). After stirring for 5 min, HOAc (75 mg) was charged and stirred for 20 min. Then $NaBH_3CN$ (93 mg, 1.48 mmol) was added and stirred for 1 h. The reaction mixture was quenched with satd $NaHCO_3$, extracted with ethyl acetate, and dried on $MgSO_4$. The solvent was removed, and **16d** was purified by column chromatography using hexane/ethyl acetate gradient elution system as a white solid. Yield: 53.1%. Mp (hexane) 81–83 °C. 1H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, 2H, $J=8.4$ Hz), 7.24 (d, 2H, $J=8.4$ Hz), 7.15 (br, 1H), 7.15 (br, 1H), 3.72 (s, 2H), 3.27 (s, 2H), 3.26–3.22 (m, 2H), 2.17 (s, 3H), 1.51–1.47 (m, 2H), 1.25 (s, 26H), 0.87 (t, 3H, $J=6.6$ Hz). MS (ESI) m/z 468.4 $[M+Na]^+$, 444.1 $[M-H]^-$. Anal. Calcd for $C_{29}H_{51}N_3O_2S$: C, 72.76; H, 10.63; N, 9.43. Found: C, 73.00; H, 10.77; N, 9.35.

6. Biology

6.1. Cell culture and cytotoxicity assay of melanoma

We examined the antiproliferative activity of ATCAA analogues in two human melanoma cell lines (A375 and WM-164) and one mouse melanoma cell line (B16-F1). We used activity on fibroblast cells as a control to determine the selectivity of these compounds against melanoma. A375 cells and B16-F1 cells were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). WM-164 cells were derived from metastatic melanoma tumors and were a gift from Dr. Meenhard Herlyn (Wistar Institute, Philadelphia, PA, USA). Human dermal fibroblast cells were purchased from Cascade Biologics, Inc., Portland, OR, USA. All cell lines were cultured in DMEM (Cellgro Mediatech, Inc., Herndon, VA, USA), supplemented with 5% FBS (Cellgro Mediatech), 1% antibiotic/antimycotic mixture (Sigma-Aldrich, Inc., St. Louis, MO, USA), and bovine insulin (5

µg/mL; Sigma-Aldrich). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. Standard sulforhodamine B assay was used. Cells were exposed to a wide range of concentrations for 48 h in round-bottomed 96-well plates. Cells were fixed with 10% trichloroacetic acid and washed five times with water. After cells were air-dried overnight and stained with SRB solution, total proteins were measured at 560 nm with a plate reader. IC₅₀ (i.e., concentration that inhibited cell growth by 50% of no treatment controls) values were obtained by nonlinear regression analysis with GraphPad Prism (GraphPad Software, San Diego, CA).

6.2. Cell culture and cytotoxicity assay of prostate cancer

We examined the antiproliferative activity of ATCAA analogues in four human prostate cancer cell lines (LNCaP, DU 145, PC-3, and PPC-1). We used LPL receptor-negative RH7777 cells as a control to determine the selectivity of these compounds. LNCaP, PC-3, DU 145, and RH7777 cells were purchased from ATCC. Dr. Mitchell Steiner at the University of Tennessee Health Science Center kindly provided PPC-1 cells. All prostate cancer cell lines were cultured in RPMI1640 (Cellgro Mediatech), supplemented with 10% FBS (Cellgro Mediatech). RH7777 cells were cultured in DMEM medium with 10% FBS. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. 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. 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 IC₅₀ values were obtained by nonlinear regression analysis using WinNonlin (Pharsight Corporation, Mountain View, CA).

6.3. Cell cycle analysis

Flow cytometry analysis was performed as described elsewhere²⁵ to study cell cycle phase distribution. Briefly, A375 and LNCaP cells (4×10^6) were seeded in each 10-cm dish; A375 and LNCaP cells were synchronized using 0.5% charcoal-stripped DMEM and RPMI medium for 72 h, respectively. After that, the medium was changed to the media containing 10% FBS with different concentrations (0, 1, 5, 10 and 20 µM) of testing compounds. Cells were incubated for additional 24 h. All cells were collected by trypsinization. Cell pellets were washed by PBS. The supernatant was discarded, and the pellets were fixed in ice-cold 70% ethanol at 4 °C overnight. Ethanol was removed by centrifugation and cell pellets were washed with PBS twice. 1 mL of PBS containing 100 µg/mL RNAase A was then added and cell pellets were incubated for 1 h. Cells were centrifuged and re-suspended in 1 mL of PBS containing propidium iodide (50 µg/mL). Cell cycle analysis was conducted with a FACS Calibur Autometer (Beckton Dickinson, San Diego, CA, USA). Data were analyzed and graphs were prepared using the MODFIT 2.0 program (Verity Software House, Topsham, ME, USA).

6.4. FLIPR intracellular calcium mobilization assay

Chem-1 cells (Chemicon Int., Inc., Billerica, MA) were stable-transfected with LPA₁, LPA₃, and LPA₅, respectively. Cells are maintained in DMEM/10% FBS/100 U/mL penicillin. Cells are passaged by washing with Ca²⁺ and Mg²⁺-free HBSS (10 mL/T75) and incubated with 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) for 5–10 min at 37 °C. Cells are loaded with Fluo-4 NW, and the assay was read for 180 s using the FLIPR^{tetra}. The signal is proportional to [Ca²⁺]_i and thus can be used to quantify differences in calcium mobilization in ATCAA-treated and control cells. Percentage activations were determined upon initial addition of **3ad** followed by 10-min incubation at 25 °C. To study antagonism of ATCAA on LPAs, cells were incubated with **3ad** for 10 min at 25 °C. Following compound incubation, reference agonists (oleoyl-LPA) were added at EC80 to determine percentage inhibition. Compound **3ad** was plated in an eight-point, fourfold serial dilution series with a top concentration of 10 μM. The concentration described here reflects the final concentration of the compound during the antagonist assay.

6.5. Colony-formation assay

To measure colony formation and growth of melanoma, A375 cells were plated at a colony-forming density (2000 cells per well on 6-well plates). Cells were grown in DMEM supplemented with FBS (Atlanta Biologicals, Lawrenceville, GA) and an antibiotic-antimycotic solution (Sigma, St. Louis, MO) at 37 °C in an atmosphere of 95% air and 5% CO₂.²⁷ Cells were treated with compounds **1b** and **3ad** at different concentrations (2, 20, and 100 μM). Compounds were added to the medium from 1 mM DMSO stock solutions, and corresponding dilution of DMSO was used as negative (vehicle) control. Cells were grown for 14 days, and colonies were stained with 0.1% crystalline blue for 30 min and rinsed with distilled water to remove excess dye. Plates were photographed, and the number of colonies was measured by Artek 880 Automated Colony Counter (Artek Systems Corporation, Farmingdale, NY).

6.6. Formulation for in vivo studies

Compound **1b** was dissolved into a co-solvent system that was composed of 80% Tween 80 (Sigma-Aldrich, St. Louis, MO) and 20% Captex 200 (Abitec Corporation, Columbus, OH). Dacarbazine (DTIC) (Sigma-Aldrich, St. Louis, MO) was dissolved in saline solution.

6.7. Animals

Male athymic nude mice age 4–5 weeks were purchased from Harlan Laboratories (Harlan Laboratories, Indianapolis, IN, USA). The laboratory housing the animals met all Association for Assessment and Accreditation and Laboratory Animal Care specifications. All of the procedures were conducted in accordance with the guidelines of our Institutional Animal Care and Use Committee.

6.8. In vivo evaluation of antitumor efficacy

Logarithmic growth phase A375 cells were prepared in FBS-free DMEM medium (Cellgro Mediatech) at a concentration of 5×10^7 viable cells/mL and placed on ice. The cell suspension was mixed with BD Matrigel (BD Biosciences, Waltham, MA, USA) at a 1:1

ratio. This cell suspension (100 μ L) was injected subcutaneously in the right dorsal flank of each mouse. When tumor sizes reached about 150 mm³, about seven days after cell inoculation, all mice bearing tumors were divided into control and treatment groups based on tumor size ($n = 8$ per group). Each group had similar average tumor size. Mice in control groups were injected intraperitoneally with 50 μ L vehicle solution only (negative control) or DTIC at 60 mg/kg (positive control) once daily. Tumor volume was measured twice weekly with a Traceable® electronic digital caliper (Fisher Scientific, Inc., Pittsburgh, PA) and calculated using the formula $a \times b^2 \times 0.5$, where a and b represented the larger and smaller diameters, respectively.³² Tumor volume was expressed as cubic millimeters. Data were expressed as mean $\pm$ SE for each group and plotted as a function of time. Percentage tumor reduction at the conclusion of the experiment (22 days after initiating treatment) was calculated from the formula $100 \times [(T - T_0)/(C - C_0)]$, where T represents mean tumor volume of a treated group on a specific day, T_0 represents mean tumor volume of the same group on the first day of treatment, C represents mean tumor volume of a control on a specific day, and C_0 represents mean tumor volume of the same group on the first day of treatment.³³ Animal activity and average body weight of each group were monitored during the entire experiment period to assess compound toxicity. At the end of treatment, all mice were euthanized by CO₂ followed by cervical dislocation, and tumors were harvested for further studies.

NCI-60 cell line cytotoxicity data of **1a** and **1b** are available via the internet at <http://pubs.acs.org>.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

This research was supported by funds from Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center; the Van Vleet Endowed Professorship; Division of Pharmaceutics, The Ohio State University; USPHS grant 1R15CA125623, and University of Tennessee Technology Maturation Grant. Zhao Wang acknowledges the support from the Alma & Hal Reagan Fellowship. We thank Dr. George Wood's group in the College of Pharmacy, University of Tennessee Health Science Center, for their initial help with formulations for compound **1b**; Dr. Dave Dickason and Dr. Tai Ahn at GTx, Inc., for their help in developing drug formulations in this study; Dr. David Arm-bruster at the University of Tennessee Health Science Center for editorial assistance; and Dr. David Hamilton at the University of Tennessee Health Science Center animal facility for assistance in developing the approved animal protocols.

Abbreviations:

ATCAA	2-arylthiazolidine-4-carboxylic acid amide
ATCAs	2-arylthiazolidine-4-carboxylic acids
CDDP	cisplatin
DTIC	dacarbazine
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide

DMAP	4-dimethylaminopyridine
DMEM	Dulbecco's modified Eagle medium
EDCI	3-ethyl-1(<i>N,N</i> -dimethyl)aminopropylcarbodiimide
FBS	fetal bovine serum
FLIPR	fluorescence imaging plate reader
GPCR	G-protein-coupled receptors
HBSS	Hank's buffered salt solution
HOBt	1-hydroxybenzotri-azole
LPA	lysophosphatidic acid
NOE	nuclear Overhauser effect
Oleoyl-LPA	1-oleoyl lysophosphatidic acid
SAR	structure-activity relationship
SRB	sulforho-damine B

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Figure 1.
Structures of LPA and ATCAA.

Figure 2.

(A) Expanded ¹H NMR spectrum of two diastereomers of **1b** (ratio 7:3) in CDCl₃; (B) 1D NOE from irradiation of C4-H of the major isomer at 4.33 ppm, no NOE observed at 5.32 ppm; (C) 1D NOE from irradiation of C4-H of minor isomer at 3.90 ppm, shows strong NOE at 5.58 ppm. Thus, protons at C2-H and C4-H are 'cis-position' in the minor isomer.

Figure 3. Amide carbon chain length relative to IC₅₀ in prostate cancer cell lines (average IC₅₀ of DU 145, PC-3, LNCaP, and PPC-1 cells) and RH7777 cell line.

Figure 4.

Flow cytometric analysis of selected ATCAA compounds **3ad** and **1b**. Effect of selected compounds on cell cycle progression of prostate cancer LNCaP and melanoma A375 cells was examined according to the procedure described in Section 5. Sub-G1 phase accumulation was induced by ATCAA compounds.

Figure 5. FLIPR intracellular calcium mobilization assay in agonist mode. Calcium flux in LPA1-, LPA3-, and LPA5-expressing Chem-1 cell lines induced by oleoyl-LPA. Oleoyl-LPA increased $[Ca^{2+}]_i$ in a dose-dependent manner; **3ad** and LPA antagonist Ki15425 did not show agonist effect.

Figure 6.

(A) Response of **3ad** and oleoyl-LPA on LPA1, LPA3, and LPA5 kinetic data. Based upon these kinetic traces exhibited by **3ad**, the relatively small Ca²⁺ flux is nonLPA receptor mediated. (B) Antagonist data of **3ad**, which did not show antagonism on LPA1, LPA3, or LPA5. Ki16425 had an IC₅₀ = 46 nM on LPA1.

Figure 7.
Compounds **3ad** and **1b** inhibit melanoma colony formation.

Figure 8.

Growth of A-375 tumor treated with compound **1b** or DTIC. (A) in vivo results of treatment of **1b** and DTIC on nude mice bearing A375 tumors compared with control group. (B) Average body weight change in control and treatment groups.

Figure 9.
SAR relationship of ATCAA.

Scheme 1.

Synthesis of ATCAA. Reagents and conditions: (a) C_2H_5OH , H_2O (70–99%); (b) Boc₂O, 1 N NaOH, 1,4-dioxane, H_2O (89–97%); (c) EDCI, HOBt, Et₃N, R^2R^3NH , CH_2Cl_2 ; (d) TFA, CH_2Cl_2 ; (e) CH_3COCl , pyridine, CH_2Cl_2 (74%); (f) DCC, DMAP, 0.3 equiv (1*S*)-(+)-10 camphorsulfonic acid, $C_{16}H_{33}OH$ (94%); (g) EDCI, HOBt, NMM, $HNCH_3OCH_3$, CH_2Cl_2 (71%); (h) LAH, THF (84%); (i) R^2NH_2 , $NaBH_3CN$, HOAc; (j) LAH, THF, 0 °C to rt or B_2H_6 , THF, rt to reflux.

Scheme 2.

Reagents: (a) $\text{C}_2\text{H}_5\text{OH}$, H_2O (78%); (b) Fmoc-Cl, Et_3N , CH_2Cl_2 (99%); (c) EDCl, HOBT, Et_3N , R^2NH_2 , CH_2Cl_2 (71%); (d) CH_3COCl , CH_3OH (48%); (e) DBU, CH_2Cl_2 (46–66%)
 (f) **15a** $\text{CH}_3\text{SO}_2\text{Cl}$, pyridine (80%); **15b** ClCH_2COCl , pyridine (51%); **15c** NaOCN, HOAc, CH_3OH , H_2O (88%).

Scheme 3.

Reagents: (a) Boc₂O, 1 N NaOH; (b) EDCI, HOBT, C₁₆H₃₃NH₂; (c) CF₃COOH; (d) *N*-(4-formyl-phenyl)-acetamide, MeOH, (e) NaBH₃CN.

Table 1

Antiproliferative effects of ATCAA in three melanoma cell lines and fibroblast cell line

ID	X	R ¹	R ²	R ³	R ⁴	B16-F1	A375	WM-164	Fibroblast	Aver. ^a	Ratio ^b
3aa	CON	3,4,5-Trimethoxy	<i>n</i> -C ₁₈ H ₃₇	H	H	5.9 ± 0.4	4.6 ± 0.2	3.0 ± 0.1	4.7 ± 0.4	4.5	1.0
3ab	CON	3,4,5-Trimethoxy	(<i>Z</i>)-Octadec-8-enyl	H	H	4.7 ± 0.1	2.4 ± 0.1	1.3 ± 0.1	4.8 ± 0.3	2.8	1.7
3ac	CON	3,4,5-Trimethoxy	(<i>E</i>)-Octadec-8-enyl	H	H	3.2 ± 0.2	1.8 ± 0.1	1.1 ± 0.1	4.0 ± 0.3	2.0	2.0
3ad	CON	3,4,5-Trimethoxy	<i>n</i> -C ₁₆ H ₃₃	H	H	1.6 ± 0.2	1.4 ± 0.2	0.7 ± 0.1	2.4 ± 0.4	1.2	2.0
3ba	CON	3,4-Dimethoxy	<i>n</i> -C ₁₈ H ₃₇	H	H	14.3 ± 0.5	6.9 ± 0.3	2.7 ± 0.2	20.3 ± 1.1	8.0	2.5
3bb	CON	3,4-Dimethoxy	(<i>Z</i>)-Octadec-8-enyl	H	H	3.3 ± 0.2	1.6 ± 0.1	1.2 ± 0.1	18.3 ± 0.8	2.0	9.0
3bc	CON	3,4-Dimethoxy	(<i>E</i>)-Octadec-8-enyl	H	H	3.2 ± 0.3	1.4 ± 0.1	1.1 ± 0.1	16.3 ± 0.7	1.9	8.6
3ca	CON	2-OMe	<i>n</i> -C ₁₆ H ₃₃	H	H	4.3 ± 0.2	3.0 ± 0.4	2.4 ± 0.2	7.2 ± 0.3	3.2	2.2
3da	CON	3-OMe	<i>n</i> -C ₁₈ H ₃₇	H	H	3.0 ± 0.1	1.8 ± 0.1	1.2 ± 0.1	2.5 ± 0.2	2.0	1.3
3ea	CON	4-OMe	<i>n</i> -C ₁₆ H ₃₃	H	H	2.3 ± 0.3	1.5 ± 0.1	1.0 ± 0.1	8.1 ± 0.5	1.6	5.1
3fa	CON	4-NMe ₂	<i>n</i> -C ₁₆ H ₃₃	H	H	6.7 ± 0.5	1.8 ± 0.1	1.5 ± 0.1	21.0 ± 3.1	3.3	6.3
3ga	CON	2-NHAc	<i>n</i> -C ₁₆ H ₃₃	H	H	8.0 ± 0.4	9.3 ± 0.6	3.9 ± 0.5	27.8 ± 3.6	7.1	3.9
3ha	CON	3-NHAc	<i>n</i> -C ₁₆ H ₃₃	H	H	2.2 ± 0.2	1.5 ± 0.1	1.1 ± 0.1	6.3 ± 0.4	1.6	3.9
3ia	CON	4-NHAc	<i>n</i> -C ₁₈ H ₃₇	CH ₃	H	18.9 ± 1.3	20.6 ± 2.1	10.7 ± 0.9	>100	16.7	-
31b	CON	4-NHAc	1-Adamantanylamino	H	H	96.5 ± 3.6	137.5 ± 4.2	127.5 ± 2.8	>100	120.5	-

ID	X	R ¹	R ²	R ³	R ⁴	B16-F1	A375	WM-164	Fibroblast	Aver. ^a	Ratio ^b
3ic	CON	4-NHAc	2-Adamantanyl	H	H	108.2 ± 4.6	66.0 ± 2.4	64.4 ± 3.0	>100	79.5	-
3id	CON	4-NHAc	9H-Fluoren-2-yl	H	H	3.9 ± 0.3	2.1 ± 0.1	1.7 ± 0.1	28.9 ± 1.0	2.6	11.3
3ie	CON	4-NHAc	Anthracen-2-yl	H	H	5.6 ± 0.2	3.1 ± 0.1	1.4 ± 0.1	13.5 ± 0.5	3.4	4.0
3if	CON	4-NHAc	4-Biphenyl	H	H	5.7 ± 0.3	6.2 ± 0.4	4.1 ± 0.2	33.4 ± 3.5	5.3	6.3
3ig	CON	4-NHAc	2-Benzothiazolyl	H	H	55.3 ± 2.9	45.9 ± 3.7	>100	>100	50.6	-
3ih	CON	4-NHAc	(Z)-Hexadec-9-enyl	H	H	2.1 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	14.0 ± 0.9	2.0	7.0
3ii	CON	4-NHAc	Nonadec-10-ynyl	H	H	1.6 ± 0.1	2.7 ± 0.2	1.3 ± 0.1	7.0 ± 0.3	1.9	3.8
3jb	CON	H	(Z)-Octadec-8-enyl	H	H	6.0 ± 0.5	4.9 ± 0.7	2.4 ± 0.1	11.6 ± 0.8	4.4	2.6
3jc	CON	H	(E)-Octadec-8-enyl	H	H	10.1 ± 0.8	5.3 ± 0.4	2.4 ± 0.2	12.4 ± 1.1	5.9	2.1
4	CON	4-NHAc	<i>n</i> -C ₁₆ H ₃₃	H	Ac	15.0 ± 0.8	12.6 ± 0.5	20.4 ± 0.8	57.7 ± 1.3	16	3.6
5	COO	4-NHAc	<i>n</i> -C ₁₆ H ₃₃	H	H	41.9 ± 3.1	28.0 ± 2.1	18.5 ± 0.4	>100	29.5	-
8a	CH ₂ N	H	<i>n</i> -C ₁₆ H ₃₃	H	H	4.7 ± 0.2	13.0 ± 0.3	4.9 ± 0.1	60 ± 1.2	7.5	8.0
8b	CH ₂ N	4-NHAc	<i>n</i> -C ₁₆ H ₃₃	H	H	11.9 ± 0.4	14.4 ± 0.3	3.2 ± 0.1	84.5 ± 2.5	9.8	8.6
15a	CON	4-NHCO ₂ CH ₃	<i>n</i> -C ₁₆ H ₃₃	H	H	113.6 ± 2.1	50.6 ± 1.6	18.6 ± 1.0	>100	60.9	-
15b	CON	4-NHCOCH ₂ Cl	<i>n</i> -C ₁₆ H ₃₃	H	H	93.3 ± 3.6	20.4 ± 1.3	5.7 ± 0.2	25.4 ± 1.3	39.8	0.6
15c	CON	4-NHCONH ₂	<i>n</i> -C ₁₆ H ₃₃	H	H	6.5 ± 0.6	3.6 ± 0.3	1.7 ± 0.1	7.2 ± 0.4	3.9	1.8
1a	CON	H	<i>n</i> -C ₁₈ H ₃₇	H	H	15.5 ± 0.6	15.0 ± 0.5	4.4 ± 0.2	29.8 ± 2.1	11.6	2.6
1b	CON	4-NHAc	<i>n</i> -C ₁₆ H ₃₃	H	H	2.2 ± 0.3	2.1 ± 0.2	1.1 ± 0.1	16.0 ± 2.5	1.8	8.9

ID	X	R ¹	R ²	R ³	R ⁴	IC ₅₀ ± SEM(μM)				Aver. ^a	Ratio ^b
						B16-F1	A375	WM-164	Fibroblast		
DTIC						>100	>100	>100	>100	-	-
CDDP						>100	>100	>100	>100	-	-
Sorafenib						4.9 ± 0.3	5.4 ± 0.5	5.0 ± 0.2	15.1 ± 1.2	5.1	3.0

^a Average IC₅₀ of three melanoma cells IC₅₀s.

^b Ratio = (IC₅₀ of fibroblast cell)/(the average IC₅₀ of melanoma cell lines).

Table 2

Antiproliferative effects of ATCAA in four prostate cancer cell lines and RH7777 cell line

ID	X	R ¹	R ²	R ³	R ⁴	DU 145	PC-3	LNCaP	PPC-1	RH7777	Aver. ^a	Ratio ^b
3aa	CON	3,4,5-Trimethoxy	<i>n</i> -C ₁₈ H ₃₇	H	H	4.1 ± 0.5	4.9 ± 0.4	1.6 ± 0.2	1.0 ± 0.1	14.0 ± 1.4	2.9	4.8
3ab	CON	3,4,5-Trimethoxy	(<i>Z</i>)-Octadec-8-enyl	H	H	1.2 ± 0.1	1.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	8.4 ± 0.8	1.0	8.6
3ac	CON	3,4,5-Trimethoxy	(<i>E</i>)-Octadec-8-enyl	H	H	0.9 ± 0.1	1.2 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	6.8 ± 0.7	0.8	9.1
3ba	CON	3,4-Dimethoxy	<i>n</i> -C ₁₈ H ₃₇	H	H	5.5 ± 1.7	4.1 ± 0.5	2.4 ± 0.4	0.9 ± 0.1	11.8 ± 0.6	3.2	3.7
3bb	CON	3,4-Dimethoxy	(<i>Z</i>)-Octadec-8-enyl	H	H	2.0 ± 0.2	2.6 ± 0.3	1.3 ± 0.1	0.6 ± 0.1	10.0 ± 0.2	1.6	6.2
3bc	CON	3,4-Dimethoxy	(<i>E</i>)-Octadec-8-enyl	H	H	1.5 ± 0.2	2.0 ± 0.2	0.8 ± 0.1	0.3 ± 0.1	9.4 ± 1.0	1.2	8.2
3ca	CON	2-OMe	<i>n</i> -C ₁₆ H ₃₃	H	H	6.5 ± 0.9	7.4 ± 1.1	3.6 ± 0.3	1.7 ± 0.1	17.2 ± 0.3	4.8	3.6
3da	CON	3-OMe	<i>n</i> -C ₁₆ H ₃₃	H	H	1.1 ± 0.1	1.0 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	3.6 ± 0.1	0.7	5.1
3ea	CON	4-OMe	<i>n</i> -C ₁₆ H ₃₃	H	H	2.2 ± 0.2	2.7 ± 0.3	1.1 ± 0.1	0.4 ± 0.1	8.4 ± 1.1	1.6	5.3
3fa	CON	4-NMe ₂	<i>n</i> -C ₁₆ H ₃₃	H	H	3.2 ± 0.3	6.0 ± 0.5	1.4 ± 0.1	0.4 ± 0.1	10.0 ± 1.6	2.8	3.6
3ga	CON	2-NHAc	<i>n</i> -C ₁₆ H ₃₃	H	H	4.6 ± 0.5	2.7 ± 0.5	3.3 ± 0.4	1.5 ± 0.2	13.2 ± 1.8	3.0	4.4
3ha	CON	3-NHAc	<i>n</i> -C ₁₆ H ₃₃	H	H	2.3 ± 0.2	1.6 ± 0.2	1.4 ± 0.2	0.5 ± 0.2	4.9 ± 0.4	1.5	3.4
3ia	CON	4-NHAc	<i>n</i> -C ₁₈ H ₃₇	CH ₃	H	3.9 ± 0.4	3.7 ± 0.2	1.8 ± 0.1	0.8 ± 0.1	9.2 ± 0.9	2.6	3.6
3ib	CON	4-NHAc	1-Adamantanylyl	H	H	>20	>20	5.7 ± 0.9	>20	>20	5.7	—
3ic	CON	4-NHAc	2-Adamantanylyl	H	H	>20	>20	9.7 ± 1.0	>20	>20	9.7	-
3id	CON	4-NHAc	9 <i>H</i> -Fluoren-2-yl	H	H	1.9 ± 0.3	2.1 ± 0.1	3.5 ± 0.7	1.6 ± 0.1	5.4 ± 0.7	2.3	2.4

ID	X	R ¹	R ²	R ³	R ⁴	DU 145	PC-3	LNCaP	PPC-1	RH7777	Aver. ^a	Ratio ^b
3e	CON	4-NHAc	Anthracen-2-yl	H	H	0.9 ± 0.1	0.8 ± 0.1	1.7 ± 0.3	0.7 ± 0.1	2.6 ± 0.4	1.0	2.5
3f	CON	4-NHAc	4-Biphenyl	H	H	5.0 ± 0.5	7.6 ± 0.6	3.5 ± 0.3	2.9 ± 0.1	9.5 ± 0.8	4.8	2.0
3g	CON	4-NHAc	2-Benzothiazolyl	H	H	>20	>20	>20	>20	>20	-	-
3h	CON	4-NHAc	(Z)-Hexadec-9-enyl	H	H	2.4 ± 0.2	2.5 ± 0.2	1.8 ± 0.1	1.1 ± 0.1	7.6 ± 0.8	2.0	3.9
3i	CON	4-NHAc	Nonadec-10-ynyl	H	H	1.8 ± 0.1	2.1 ± 0.6	2.0 ± 0.4	0.7 ± 0.2	6.6 ± 1.3	1.7	4.0
3j	CON	H	(Z)-Octadec-8-enyl	H	H	1.8 ± 0.2	2.4 ± 0.2	1.6 ± 0.1	1.0 ± 0.1	8.3 ± 0.9	1.7	4.9
3j	CON	H	(E)-Octadec-8-enyl	H	H	1.5 ± 0.1	2.1 ± 0.2	1.4 ± 0.1	0.6 ± 0.1	8.9 ± 0.9	1.4	6.5
4	CON	4-NHAc	n-C ₁₆ H ₃₃	H	Ac	14.9 ± 2.2	9.0 ± 1.3	10.6 ± 0.6	6.8 ± 0.8	13.0 ± 3.0	10.3	1.3
5	COO	4-NHAc	n-C ₁₆ H ₃₃	H	H	>20	>20	>20	>20	>20	-	-
8a	CH ₂ N	H	n-C ₁₆ H ₃₃	H	H	3.1 ± 0.3	3.4 ± 0.3	3.9 ± 0.4	2.5 ± 0.2	6.2 ± 0.4	3.2	1.9
8b	CH ₂ N	4-NHAc	n-C ₁₆ H ₃₃	H	H	4.7 ± 0.3	4.5 ± 0.5	4.7 ± 0.5	2.4 ± 0.2	7.9 ± 0.6	4.1	1.9
13a	CON	4-NH ₂	n-C ₁₂ H ₂₅	H	H	2.4 ± 0.1	2.9 ± 0.1	2.0 ± 0.2	1.2 ± 0.1	7.3 ± 0.8	2.1	3.4
13b	CON	4-NH ₂	n-C ₁₆ H ₃₃	H	H	2.6 ± 0.1	2.4 ± 0.1	1.5 ± 0.1	0.6 ± 0.1	9.4 ± 0.1	1.8	5.3
15a	CON	4-NHSO ₂ CH ₃	n-C ₁₆ H ₃₃	H	H	>20	>20	4.8 ± 1.0	1.1 ± 0.1	>20	2.9	-
15b	CON	4-NHCOCH ₂ C1	n-C ₁₆ H ₃₃	H	H	0.7 ± 0.2	0.9 ± 0.2	2.0 ± 0.2	0.4 ± 0.1	9.4 ± 0.9	1.0	9.4
15c	CON	4-NHCONH ₂	n-C ₁₆ H ₃₃	H	H	3.2 ± 0.3	2.5 ± 0.4	1.8 ± 0.2	0.5 ± 0.2	6.7 ± 0.6	2.0	3.4
1a	CON	H	n-C ₁₈ H ₃₇	H	H	10.8 ± 1.8	10.0 ± 0.7	4.2 ± 0.4	2.4 ± 0.2	>20	6.9	-
1b	CON	4-NHAc	n-C ₁₆ H ₃₃	H	H	1.7 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	0.4 ± 0.1	6.8 ± 0.7	1.1	6.3

Average IC₅₀ of four prostate cells IC₅₀s.
$$p \text{ Ratio} = (\text{IC}_{50} \text{ of RH7777 cell}) / (\text{average IC}_{50} \text{ of prostate cell lines}).$$

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Table 3

Antiproliferative effects of thiazolidine ring-open compounds against two melanoma cell lines and fibroblast cell line

ID	IC ₅₀ ± SEM (μM)		
	A375	WM-164	Fibroblast
16a	12.9 ± 1.3	14.4 ± 0.8	61.2 ± 3.6
16b	108.5 ± 4.8	144.9 ± 5.2	>100
16c	15.1 ± 1.1	12.8 ± 2.1	16.4 ± 1.2
16d	11.9 ± 0.6	10.2 ± 0.5	24.0 ± 1.3
3ja	ND ^a	0.7 ± 0.1	2.2 ± 0.2
1b	2.1 ± 0.2	1.1 ± 0.1	16.0 ± 2.5

^aND = not determined

Table 4

Antiproliferative effects of different chain length amides against prostate cells and R7777 cells

Compounds	Configuration at 4-position	R	DU 145	PC-3	LNCaP	PPC-1	RH7777
	3ah (4 <i>R</i>)	<i>n</i> -C ₈ H ₁₇	17.3 ± 1.4	>20	2.7 ± 0.7	14.0 ± 0.5	>20
	3ag (4 <i>R</i>)	<i>n</i> -C ₁₀ H ₂₁	4.1 ± 0.3	4.4 ± 0.3	2.2 ± 0.3	2.1 ± 0.1	6.0 ± 0.8
	3af (4 <i>R</i>)	<i>n</i> -C ₁₂ H ₂₅	2.8 ± 0.1	2.9 ± 0.1	1.4 ± 0.1	1.0 ± 0.1	6.5 ± 0.1
	3ae (4 <i>R</i>)	<i>n</i> -C ₁₄ H ₂₉	2.2 ± 0.1	2.6 ± 0.1	2.0 ± 0.3	0.6 ± 0.1	6.1 ± 0.6
	3ai (4 <i>S</i>)	<i>n</i> -C ₁₄ H ₂₉	2.1 ± 0.1	2.9 ± 0.1	1.9 ± 0.1	0.7 ± 0.1	5.8 ± 0.8
	3ad (4 <i>R</i>)	<i>n</i> -C ₁₆ H ₃₃	1.7 ± 0.1	1.8 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	7.2 ± 0.1
	3aa (4 <i>R</i>)	<i>n</i> -C ₁₈ H ₃₇	4.1 ± 0.7	4.9 ± 0.4	1.6 ± 0.2	1.0 ± 0.1	14.0 ± 0.5
	3ab (4 <i>R</i>)	(<i>Z</i>)-Octadec-8-enyl	1.2 ± 0.1	1.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	8.4 ± 0.8
	3ac (4 <i>R</i>)	(<i>E</i>)-Octadec-8-enyl	0.9 ± 0.1	1.2 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	6.8 ± 0.7