Discovery of 4-Substituted Methoxybenzoyl-aryl-thiazole as Novel Anticancer Agents: Synthesis, Biological Evaluation, and Structure−Activity Relationships

Yan Lu,[†] Chien-Ming Li,[‡] Zhao Wang,[†] Charles R. Ross, II,[§] Jianjun Chen,[†] James T. Dalton,[‡] Wei Li,[†] and Duane D. Miller*,[†]

Department of Pharmaceutical Sciences, University of Tennessee, Health Science Center, Memphis, Tennessee 38163, Division of Pharmaceutics, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, St. Jude Children's Research Hospital, Memphis, Tennessee 38103

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A series of 4-substituted methoxybenzoyl-aryl-thiazoles (SMART) have been discovered and synthesized as a result of structural modifications of the lead compound 2-arylthiazolidine-4-carboxylic acid amides (ATCAA). The antiproliferative activity of the SMART agents against melanoma and prostate cancer cells was improved from μM to low nM range compared with the ATCAA series. The structure−activity relationship was discussed from modifications of "A", "B", and "C" rings and the linker. Preliminary mechanism of action studies indicated that these compounds exert their anticancer activity through inhibition of tubulin polymerization.

Introduction

In 2008, about 565,650 Americans are expected to die of cancer, more than 1500 people a day. Cancer is the second most common cause of death in the U.S., exceeded only by heart disease. In the U.S., cancer accounts for 1 of every 4 deaths. The 5-year relative survival rate for all cancer patients diagnosed in 1996−2003 is 66%, up from 50% in 1975−1977.[1] The improvement in survival reflects progress in diagnosing at an earlier stage and improvements in treatment. Discovering highly effective anticancer agents with low toxicity is a goal of our research.

We have recently discovered 2-aryl-thiazolidine-4-carboxylic acid amides (ATCAA[a], Figure 1) as potent cytotoxic agents for both prostate cancer and melanoma.[2−6] ATCAA was designed from lysophosphatidic acid (LPA) structure with a lipid chain in order to inhibit guanine-binding protein-coupled receptor (GPCR) signaling, which was involved in proliferation and survival of prostate cancer.[7−10] The most potent compounds in ATCAA-1 derivatives could inhibit prostate cancer cells with an average IC_{50} in the range from 0.7 to 1.0 μM and average IC_{50}s against melanoma cells were 1.8−2.6 μM.[2] (2*RS*,4*R*)-2-Phenyl-thiazolidine-4-carboxylic acid hexadecylamide (ATCAA-1) was sent to the U.S. National Cancer Institute 60 human tumor cell line anticancer drug screen (NCI-60). Results from NCI-60 assay showed that compound ATCAA-1 could inhibit growth of all nine types of cancer cells with IC_{50} in the range from 0.124 μM (leukemia, CCRF-CEM) to 3.81 μM (non-small cell lung cancer, NCI-H522). SAR studies of ATCAA indicated that replacement of the lipid chain with a bulky aromatic ring in the 4-amide position of ATCAA-2 attached to the thiazolidine ring still kept the antiproliferative activity.[11] This finding afforded us a new point to replace the fatty amide chain with a number of aromatic groups, which would maintain the cytotoxicity. With further investigation of ATCAA-2 analogues, structure modifications were made on thiazolidine ring and 4-carboxylic amide linker. Thus, substituted methoxybenzoyl-aryl-thiazole (SMART) compounds were discovered and showed highly improved growth inhibition on tested cancer cells in vitro.

In this paper, we described a series of the SMART agents with general structure as showed in Figure 1. The SMART agents have a structure containing three conjugated aromatic rings ("A", "B", and "C" rings, respectively) with a ketone linkage between "B" and "C" rings. Thiazole was introduced in "B" ring instead of thiazolidine ring in ATCAA. The linker between "B" and "C" rings was modified from an amide to carbonyl group. The "C" ring was characterized by the presence of differently substituted phenyl groups, in particular, the 3,4,5-trimethoxy substituted phenyl at "C" ring played an important role of antiproliferative activity against melanoma and prostate cancer. Synthesis, SAR studies, biological evaluation, and the anticancer mechanism of the SMART analogues was undertaken and reported in this paper.

Chemistry. The general synthesis of the ATCAA and SMART analogues are shown in Schemes 1−3. To prepare ATCAA compounds **2a**−**b**, L-cysteine was allowed to react with appropriate benzaldehydes in ethanol and water at ambient temperature to give cyclized (2*RS*,4*R*)-2-aryl-thiazolidine-4-carboxylic acids **1**,[12] which were converted to the corresponding BOC-protected derivatives. Reaction of BOC-protected carboxylic acids with 3,4,5-trimethoxyaniline using EDCI/HOBt gave corresponding amides, which were treated with TFA to form the target compounds **2a**−**b**.

To synthesize thiazoline and thiazole series compounds **4a**−**b** and **5**, (4*R* or 4*S*)-2-phenyl-4,5-dihydro-thiazole-4-carboxylic acid **3a** and **3b** were obtained by reacting L- or D-cysteine with benzonitrile in methanol and pH 6.4 phosphate buffer solution at ambient temperature for several days.[13] Coupling reactions of **3a**−**3b** with 3,4,5-trimethoxyaniline under EDCI/HOBt conditions gave 4*R* or 4*S* amide **4a**−**4b**. Oxidation with $BrCCl_3$/DBU of **4a**−**4b** gave the same dehydrogenation thiazole product **5**.[14]

The SMART compounds were synthesized as illustrated in Scheme 3. (4*R*)-2-(Substituted phenyl)-4,5-dihydro-thiazole-4-carboxylic acids **3** were synthesized according the similar preparation of **3a** and **3b** from L-cysteine with appropriate

* To whom correspondence should be addressed. Phone: (901) 448-6026. Fax: (901) 448-3446. E-mail: dmiller@utmem.edu.
† University of Tennessee.
‡ The Ohio State University.
§ St. Jude Children's Research Hospital.

[a] Abbreviations: ATCAA, 2-aryl-thiazolidine-4-carboxylic acid amides; LPA, lysophosphatidic acid; GPCR, guanine-binding protein-coupled receptor; SMART, 4-substituted methoxybenzoyl-aryl-thiazoles; SAR, structure−activity relationships.

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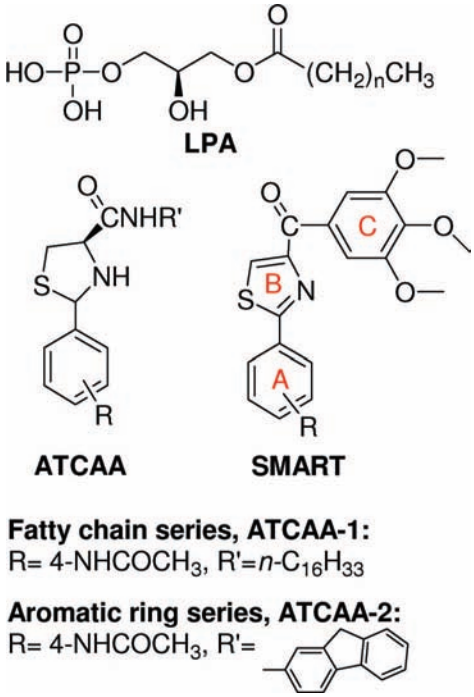


Figure 1. Structures of LPA, ATCAA, and SMART.

benzonitriles.[13,15,16] Compounds **3** can be easily converted to the corresponding Weinreb amides **6a**−**6p**[17] using EDCI/HOBt as coupling reagents. Thiazole intermediate **7** can be obtained from $BrCCl_3$/DBU dehydrogenation of **6a**−**6p**. Compound **6** or **7** was reacted with appropriate lithium reagents or Grignard reagents in anhydrous THF to give the final SMART compounds **8a**−**8z**.[17] Compound **10** was obtained by the same method. Thiazoline Weinreb amides **6a**−**6p** reacted directly with appropriate lithium reagents or Grignard reagents, and after quenching with saturated NH_4Cl solution, the mixtures of thiazoline compounds and the corresponding thiazole compounds were afforded. When thiazoline/thiazole mixtures were placed in the solvent and exposed to air under ambient atmosphere for some time (overnight to several days), the thiazoline ring spontaneously dehydrogenated to thiazoles **8a**−**8z**, which were clearly indicated by ^1H NMR, mass spectra, and elemental analysis. As an example, in solution with deuterated chloroform, mixtures of thiazoline/thiazole compounds can be slowly converted to almost pure thiazole compound **8f** after 9 days (see Figure 2). No report of autodehydrogenations was found in the literature. Most reports of thiazoline−thiazole dehydrogenations need an oxidant (MnO_2[18]), oxidase in biosynthesis,[19] or catalysts ($Hg(OAc)_2$, K_3FeCN_6).[20] The thiazoline Weinreb amide was reported to undergo dehydrogenation to form thiazole under base/solvent (NaH/MeOH, TMSOK/THF, etc.) conditions.[21] The base could abstract the acidic 4-position proton of thiazoline ring, with subsequent intramolecular attack of carbanion on the methoxy amide and release of MeO^-, followed by three-position proton elimination. We did not observe these autodehydrogenation phenomena between intermediate thiazoline amides **6a**−**6p** and thiazole amides **7**. The triple aromatic ring system formed highly stable conjugated SMART structures **8a**−**8z**, which could be a favorable reason for autodehydrogenation. X-ray crystallography demonstrated the conjugated structure of compound **8f**. Benzoic acid **8r** was prepared from the acidic hydrolysis of benzonitrile **8q** in HCl/HOAc.[22] Methyl ester **8s** was obtained by the esterification of **8r** in methanol and acetyl chloride. *para*-Amino compound **8w** was synthesized by using iron and acetic acid reduction of *para*-nitro compound **8p**.[23] 3,4,5-Trihydroxyl compound **11** was obtained using BBr_3 as demethylation reagent.[24]

Crystal Structure. The SMART compound **8f** was recrystallized from hexane and ethyl acetate, and single colorless crystals suitable for X-ray diffraction were obtained. An ORTEP drawing of **8f** with the atom labeling scheme is shown in Figure 3. The X-ray structure showed that **8f** molecule contained a conjugated system composed of three aromatic rings and a carbonyl group linker between the "B" and "C" rings as expected ("A" ring = phenyl; "B" ring = thiazole; "C" ring = 3,4,5-trimethoxyphenyl). As a result, two C−C bonds adjacent to C═O and C−C− bond between the "A" phenyl and "B" thiazole rings display (C1−C7 = 1.496(2) Å; C7−C8 = 1.492(2) Å; C10−C11 = 1.471(2) Å) shorter bond lengths than normal C−C single bond (1.54 Å) and longer than a normal C═C double bond (1.34 Å) (see Table 1). Thus conjugation of the π system is possible for "A", "B", and "C" rings and carbonyl group. The carbonyl group is nearly coplanar with the adjacent "B" thiazole ring (O−C7−C1-C6 16.2(2)°, O−C7−C8-C9 9.7(2)°).

Biological Results and Discussion. ATCAA to SMART molecules: Modifications of the "B" ring from a thiazolidine to thiazole system and the linker from an amide to a ketone. In the previous ATCAA compounds, we found the thiazolidine ring, which contained a free NH at its 3-position, was important for cytotoxicity. Once the "B" ring thiazolidine moiety was replaced by a thiazoline ring, the antiproliferative activity decreased sharply from 0.6 μM to over 50 μM on WM-164 cell line.[2] The ATCAA-1 fatty amide derivatives that was most effective against melanoma and prostate cancer cell lines were examined and shown to have an IC_{50} 0.4−2.2 μM (Table 2). Replacement of the long fatty chain with a certain aromatic bulky subsistent such as fluorene (ATCAA-2) showed inhibitory activity on both cancer cell lines (IC_{50} = 1.6−3.9 μM).[11] The fluorene group in 4-carboxylic amide position was also replaced by 3,4,5-trimethoxyphenyl group (**2**a and **2b**), but the potency against both cancer cell lines was lost. The subsequent "B" ring modification from saturated thiazolidine compound **2a** to unsaturated thiazole **5** did not show any cytotoxicity against either cancer cell line tested. But thiazoline enantiomers **4a** and **4b** (*R*-isomer and *S*-isomer showed similar antiproliferative activities) showed improved activity (IC_{50} = 3.4−38.3 μM) compared with **2a**, **2b**, and **5**. When the amide CONH linkage between the "B" ring and the "C" ring was replaced by a carbonyl linker, the mixtures of thiazoline/thiazole ketone **8f** were obtained instead of desired thiazoline ketone, because the autodehydrogenation between thiazoline and thiazole occurred (the conversion was shown in Figure 2). Surprisingly, introduction of the carbonyl group linker and thiazole led to a significant enhancement of growth inhibition of examined cancer cell lines with a low nanomolar level (**8f**, IC_{50} = 0.021−0.071 μM), which is comparable to the natural anticancer agent colchicine. Thus a series of the SMART compounds with "B" as a thiazole ring were designed and synthesized based on the discovery of **8f** and their anticancer activity was evaluated against melanoma and prostate cancer.

Modifications of the "C" Ring of the SMART Molecules. We started our investigation of the "C" position of the SMART by introducing different substituted phenyls or alkyl chain. Variation of the phenyl substituents has a remarkable change in effect on potency. The in vitro assay as shown in Table 3 gave us an interesting result, but only 3,4,5-trimethoxyphenyl in the "C" ring (**8f**) showed excellent inhibition against all cancer cells (IC_{50}= 21−71 nM, average IC_{50} = 41 nM). Compound

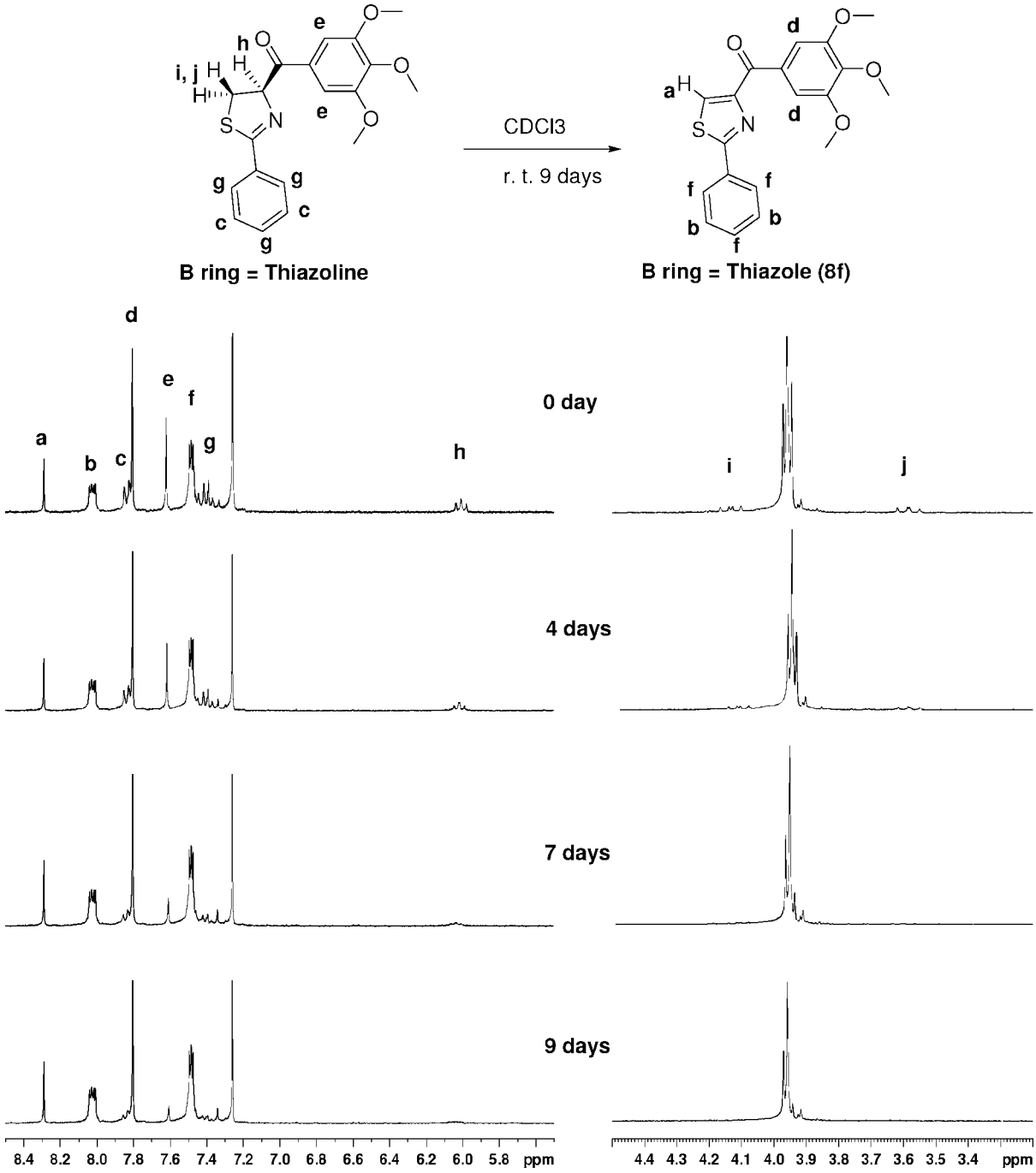


Figure 2. Autodehydrogenation from thiazoline to thiazole compound **8f**. At 0 day, NMR sample contained thiazoline and thiazole mixtures in $CDCl_3$; ratio is about 3: 2. At ninth day, thiazoline compound was almost converted to thiazole compound **8f**.

8g, with a 3,5-dimethoxyphenyl group, showed 6-fold average cytotoxicity lower than **8f** against six different cell lines (IC_{50} = 170−424 nM, calcd average IC_{50} = 261 nM). Modifications of **8f** by removal of one methoxy at *meta*-position (**8e**) or two methoxy groups (**8b**, **8c**, and **8d**) from **8f** led to a dramatic loss in activity (IC_{50} > 20 μM). Although *ortho*-substituted monomethoxy compound **8d** exhibited weak activity against a certain cell lines compared with *meta*-/*para*-MeO substituted **8c**/**8b** and dimethoxyphenyl compound **8e**, none of them showed significant potency in inhibition compared with **8f**. Similar trends were also seen in **8h** and **8j** with 2-fluorophenyl and hexadecyl in "C" ring modifications.

Modifications of the "A" Ring of the SMART Molecules. In SAR studies of the ATCAA compounds, we found that the electronic properties of substituents of the phenyl ring in the 2-position of the thiazolidine ring strongly affected the anticancer activity in ATCAA compounds—electron-withdrawing groups (EWG) on 2-phenyl gave higher activities than those with electron-donating groups (EDG).[6] We also introduced different *para*-substituted EWG and EDG on the "A" phenyl ring of the SMART molecules. From the IC_{50} value against these cancer cell lines, electronic effects of "A" ring phenyl substituents did not show clear influence on antiproliferative activity. Introduction of a weak EWG (4-F in **8n**, IC_{50}s: 6−43 nM) or weak EDG (4-CH_3 in **8k**, IC_{50}s: 5−21 nM), both increased the potency compared with **8f** (see Table 4). The replacement of the *para*-position with strong EWG such as NO_2 (**8p**), CN (**8q**), CF_3 (**8t**), or introducing strong EDG (3,4-dimethoxy) to the "A" phenyl ring (**8o**) exhibited comparable antiproliferative activity.

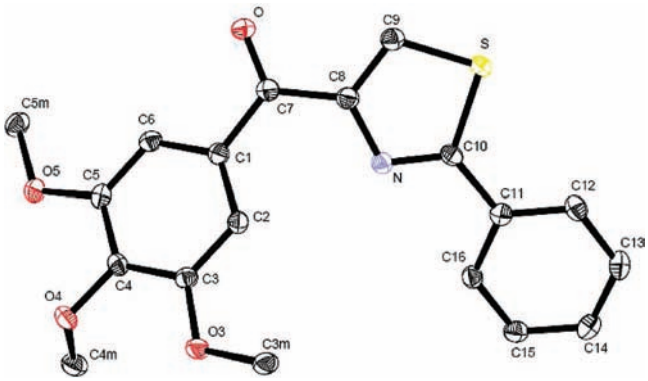


Figure 3. ORTEP drawing of **8f** with thermal ellipsoids depicted at 50% probability level.

Table 1. Selected Geometric Parameters of **8f** (Å, deg)

C1−C7	1.496(2)	O−C7−C1	120.1(2)
C7−O	1.224(2)	C8−C7−C1	121.9(2)
C7−C8	1.492(2)	C9−C8−N	115.1(2)
C8−C9	1.371(2)	C9−C8−C7	121.7(2)
C8−N	1.380(2)	N−C8−C7	123.0(2)
C9−S	1.711(2)	C8−C9−S	110.0(1)
S−C10	1.747(2)	C9−S−C10	89.6(1)
C10−N	1.303(2)	N−C10−C11	123.5(2)
C10−C11	1.471(2)	N−C10−S	113.9(1)
C2−C1−C6	121.2(2)	C11−C10−S	122.6(1)
C2−C1−C7	122.3(2)	C10−N−C8	111.4(2)
C6−C1−C7	116.4(2)	C12−C11−C10	122.3(2)
O−C7−C8	118.0(2)	C16−C11−C10	118.5(2)

To compare the effects of *ortho*-, *meta*-, and *para*-substitutions, a fluoro atom was introduced to different positions of the "A" phenyl ring (**8l**, **8m**, and **8n**). The various *o*-, *m*-, *p*-substituents did not exhibit equal activities. *p*-Fluoro substituted **8n** has the best activity for examined prostate cancer cells (6−13 nM), while *o*-fluoro substituted **8l** showed the lowest IC_{50}s (27−30 nM) against melanoma cells. **8n** has similar average IC_{50}s (33−43 nM) against melanoma compared with **8l**. But *o*-fluoro-substituted **8l** has lowest potency (IC_{50}s: 52−114 nM) among the three substituted compounds on prostate cancer cells. *Meta*-substituted compound **8m** showed lowest activity on melanoma cells (IC_{50}s: 287−304 nM) but showed moderate inhibition on prostate cancer cells (IC_{50}s: 23−46 nM).

Turning to the effects of steric hindrance group on the "A" phenyl ring substituents, we found that *p*-bromo (**8u**, IC_{50}s: 18−44 nM) caused a decrease in antiproliferative activity relative to *p*-fluoro position (**8n**, IC_{50}s: 6−12 nM) only against prostate cancer cells. Reduced activity against both cancer cell lines occurred when *p*-methyl (**8k**, IC_{50}s: 5−21 nM) was replaced with a *p*-ethyl group (**8v**, IC_{50}s: 17−70 nM).

To investigate if phenyl played an essential role at the "A" ring in cytotoxicity, we also removed phenyl at 2-thiazole position and compound **10** was obtained. This modification caused a total loss of activity compared with **8f**. The replacement of the "A" ring by pyridine (compound **8x**) had the same effect. Moreover, substituting 2-pyrimidine in "A" ring (compound **8y**) also caused a significant loss of activity (IC_{50}s: 11.8−41.0 μM). However, introducing the thiophene replacement of phenyl (**8z**) into the "A" position improved the potency calcd 1−3 fold on all examined cell lines (IC_{50}s: 9−38 nM) compared to **8f** (IC_{50}s: 21−71 nM).

Addition of Pharmaceutically Acceptable Salt Groups to the SMART Molecules. Most of the SMART compounds have good solubility in organic solvents such as $CHCl_3$, CH_2Cl_2, and DMSO. But they show poor water solubility. We designed and synthesized water-soluble salts of the SMART by introducing a hydrophilic group such as NH_2 (**8w**) and COOH (**8r**) into the "A" ring and generated the HCl or sodium salt. Another modification is replacing "A"/"C" rings in **8a** with pyridine (**8i**, **8x**, **8y**) or pyrimidine rings, which could also be converted into HCl salts. These modifications reduced the calculated LogP values (LogP = 2.74−3.90) compared with **8a** and **8f** (LogP = 4.46 and 4.08, see Table 5). Introducing *p*-amino to "A" phenyl (**8w**) is the only case to increase the antiproliferative activity (HCl salt, IC_{50}s: 11−29 nM) compared with **8f** against all cell lines. Although replacing phenyl with pyrimidine (**8y**) kept partial activity against both cancer cells, the potency range was markedly reduced from nM to μM compared with **8f**. Unfortunately, introducing COOH to the *para*-phenyl "A" ring and pyridine to the "A" or "C" rings (**8i**, **8r**, **8x**) all resulted in the total loss of the anticancer activity. A total loss of potency was seen in the methyl ester **8s** of acid **8r** against both cancer cell lines. Demethylation of compound **8f** afforded water soluble 3,4,5-trihydroxyphenyl at "C" ring compound **11**, but this demethylation results in complete loss of antiproliferative activity against all tested cancer cells, which also points out the importance of 3,4,5-trimethoxyphenyl at the "C" position.

Mechanism of Action Studies: The SMART Compounds Inhibit Tubulin Polymerization. By observing cell cycle progression in response to **8f**, a significant increase in G2/M phase arrest was detected (unpublished data). This is similar to the induction by an mitotic inhibitor, such as colchicine.[25] To investigate whether the antiproliferative activities of these compounds were related to interaction with tubulin, the SMART compound **8f** was evaluated for inhibition of polymerization of purified tubulin in a cell-free system. The results are shown in Figure 4. Compared with nontreated control, **8f** ("A" ring = phenyl, "C" ring = 3,4,5-trimethoxyphenyl) inhibits tubulin polymerization. The effect of **8f** on tubulin assembly was examined at concentrations from 0.625 μM to 20 μM. We observed that compound **8f** inhibited tubulin polymerization in a dose-dependent manner (Figure 4), with an IC_{50} value of 4.23 μM.

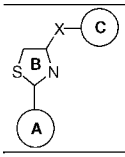
Conclusions

We have discovered a new class of simple synthetic inhibitors of tubulin polymerization, based on a 2-aryl-4-(3,4,5-trimethoxybenzoyl)-thiazole molecular skeleton, which was derived from thiazolidine ring modification of ATCAA structures. A series of the SMART compounds were synthesized. Chemical modification of different substituted aryl in "A" and "C" rings and structure−activity relationship of the SMART were investigated (Figure 5) based on biological evaluation against melanoma and prostate cancer cells in vitro. Present SAR studies revealed that 3,4,5-trimethoxyphenyl was the essential group in the "C" ring to keep excellent antitumor potency. *p*-Fluoro, *p*-NH_2, and *p*-CH_3 substituents in "A" ring will increase the activity, with no clear difference in effect on activity between EWG and EDG when "A" are substituted phenyl rings. The carbonyl linkage between "B" ring and "C" ring played an important role for the high potency. Further modification of "B" and "C" rings, carbonyl linkage, tubulin binding site studies, mechanism of action of the SMART compounds, and in vivo animal testing are currently underway.

Experimental Section

General. All reagents were purchased from Sigma-Aldrich Chemical Co., Fisher Scientific (Pittsburgh, PA), AK Scientific (Mountain View, CA), Oakwood Products (West Columbia, SC),

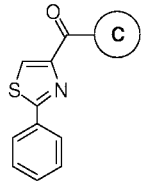
Table 2. In Vitro Inhibitory Effects of Modified ATCAA Compounds against the Proliferation of Melanoma (A 375, B16−F1) and Prostate Cancer Cells (DU145, PC-3, LNCaP, PPC-1)



	A ring	B ring[a]	C ring[b]	X	$IC_{50} \pm SEM$ (μM)					
					B16-F1	A375	DU 145	PC-3	LNCaP	PPC-1
ATCAA-1	*p*-NHAc-Ph	TZD	$C_{16}H_{33}$	CONH	2.2±0.3	2.1±0.2	1.7 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	0.4 ± 0.1
ATCAA-2	*p*-NHAc-Ph	TZD	9H-fluoren-1-yl	CONH	3.9±0.3	2.1±0.1	1.9 ± 0.3	2.1 ± 0.1	3.5 ± 0.7	1.6 ± 0.1
2a	Ph	TZD	3,4,5-trimethoxy-Ph	CONH	>100	>100	>20	>20	>20	>20
2b	3,4,5-trimethoxy-Ph	TZD	3,4,5-trimethoxy-Ph	CONH	>100	>100	>20	>20	>20	>20
4a*(4R)*	Ph	TZL	3,4,5-trimethoxy-Ph	CONH	38.3± 3.2	22.8±1.6	>20	>20	>20	5.3± 0.3
4b*(4S)*	Ph	TZL	3,4,5-trimethoxy-Ph	CONH	30.4±2.8	13.6±1.2	>20	13.2± 2.1	16.8± 1.8	3.4± 0.2
5	Ph	TZ	3,4,5-trimethoxy-Ph	CONH	>100	>100	>20	>20	>20	>20
8f	Ph	TZ	3,4,5-trimethoxy-Ph	CO	0.055 ± 0.005	0.028 ± 0.005	0.071 ± 0.004	0.021 ± 0.001	0.028 ± 0.004	0.043 ± 0.005
Colchicine					0.029 ± 0.005	0.020 ± 0.003	0.010 ± 0.002	0.011 ± 0.001	0.016 ± 0.004	0.020 ± 0.001

[a] TZD = thiazolidine, TZL = thiazoline, TZ = thiazole. [b] For **ATCAA-1**, "C" position contains a lipid chain.

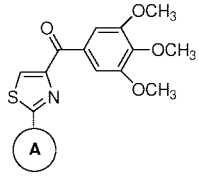
Table 3. In Vitro Growth Inhibitory Effects of Compounds **8a**−**8j** with Different "C" Rings against the Proliferation of Melanoma (A 375, B16−F1) and Prostate Cancer Cells (DU145, PC-3, LNCaP, PPC-1)



Compounds **8**		C Ring	$IC_{50} \pm SEM$ (μM)					
			B16-F1	A375	DU 145	PC-3	LNCaP	PPC-1
	8a	Ph	>100	>100	>20	>20	>20	>20
	8b	4-Methoxy-Ph	>100	>100	>20	>20	>20	>20
	8c	3-Methoxy-Ph	>100	>100	>20	>20	>20	>20
	8d	2-Methoxy-Ph	59.4 ± 21.2	70.3 ± 32.5	>20	>20	>20	>20
	8e	3, 4-Dimethoxy-Ph	>100	>100	>20	>20	>20	>20
	8f	3,4,5-Trimethoxy-Ph	0.055 ± 0.005	0.028 ± 0.005	0.071 ± 0.004	0.021 ± 0.001	0.028 ± 0.004	0.043 ± 0.005
	8g	3, 5-Dimethoxy-Ph	0.350 ± 0.2	0.170 ± 0.1	0.424 ± 0.098	0.301 ± 0.030	0.323 ± 0.041	0.242 ± 0.014
	8h	2-Fluoro-Ph	>100	>100	>20	>20	>20	>20
	8j	Hexadecyl[a]	18.6±17.5	16.0 ± 15.2	>20	>20	>20	>20

[a] Compound **8j** has a lipid chain at "C" ring position.

Table 4. In Vitro Growth Inhibitory Effects of the SMART Compounds with Different "A" Rings against the Proliferation of Melanoma (A 375, B16−F1) and Prostate Cancer Cells (DU145, PC-3, LNCaP, PPC-1)



Compounds **8**		A Ring	$IC_{50} \pm SEM$ (nM)					
			B16-F1	A375	DU 145	PC-3	LNCaP	PPC-1
	8f	Ph	55 ± 5	28 ± 5	71 ± 4	21 ± 1	28 ± 4	43 ± 5
	8k	4-Methyl-Ph	21 ± 10	11 ± 5	7 ± 1	5± 1	6 ± 1	6 ± 1
	8l	2-Fluoro-Ph	27 ± 11	30 ± 9	114 ± 3	82 ±9	53 ± 4	52 ± 3
	8m	3-Fluoro-Ph	287 ± 36	304 ± 25	35 ± 3	24 ± 2	11 ± 2	21 ± 1
	8n	4-Fluoro-Ph	43 ± 21	33 ± 14	12 ± 1	13 ± 1	6 ± 1	8 ± 1
	8o	3, 4-Dimethoxy-Ph	161 ± 29	34 ± 10	102 ± 2	69 ± 3	38 ± 6	56 ± 2
	8p	4-Nitro-Ph	56 ± 12	38 ± 9	95 ± 5	56 ± 1	39 ± 4	34 ±1
	8q	4-Cyano-Ph	53 ± 16	59 ± 24	52 ± 2	30 ± 7	15 ±4	19 ± 2
	8t	4-Trifluoromethyl-Ph	92 ± 16	23 ± 5	50 ± 5	58 ± 4	94 ± 1	76 ± 1
	8u	4-Bromo-Ph	32 ± 5	13 ± 2	21 ± 4	18 ± 3	44 ± 3	21 ± 5
	8v	4-Ethyl-Ph	70 ± 8	17± 2	31 ± 4	27 ± 4	60 ± 5	22 ± 3
	8x	4-Pyridine	>100000	>100000	>20000	>20000	>20000	>20000
	8y	2-Pyrimidine	2300 ± 860	4100 ± 740	2813 ± 92	2657 ± 40	2370 ± 85	1186 ± 22
	8z	2-Thienyl	38 ± 15	20 ± 7	22 ± 1	17 ± 2	9 ± 1	13 ± 1
	10	H[a]	>100000	>100000	>20000	>20000	>20000	>20000

[a] Compound **10** has a proton at "A" ring position.

etc. and were used without further purification. Moisture-sensitive reactions were carried under an argon atmosphere. Routine thin layer chromatography (TLC) was performed on aluminum backed Uniplates. (Analtech, Newark, DE). Melting points were measured with Fisher−Johns melting point apparatus (uncorrected). NMR spectra were obtained on a Bruker AX 300 (Billerica, MA) spectrometer or Varian Inova-500 spectrometer. Chemical shifts are reported as parts per million (ppm) relative to TMS in $CDCl_3$.

Table 5. In Vitro Growth Inhibitory Effects of Compounds Contained Hydrophilic Group Against the Proliferation of Melanoma (A 375, B16−F1) and Prostate Cancer Cells (DU145, PC-3, LNCaP, PPC-1)

	IC_{50} ± SEM (nM)						
compd	B16−F1	A375	DU145	PC-3	LNCaP	PPC-1	CLogP[a]
8i	>100000	>100000	>20000	>20000	>20000	>20000	3.55
8i·HCl	>100000	>100000	>20000	>20000	>20000	>20000	
8r	>100000	>100000	>20000	>20000	>20000	>20000	3.64
8s	>100000	>100000	>20000	>20000	>20000	>20000	3.90
8x	>100000	>100000	>20000	>20000	16630	18000	2.74
8x·HCl	>100000	>100000	>20000	>20000	>20000	>20000	
8y	2300 ± 860	4100 ± 740	2813 ± 92	2657 ± 40	2370 ± 85	1186 ± 22	3.04
8w·HCl	29 ± 10	11 ± 2	20 ± 2	12 ± 1	13 ± 1	15 ± 1	
11	>100000	>100000	>20000	>20000	>20000	>20000	3.29
8a	>100000	>100000	>20000	>20000	>20000	>20000	4.46
8f	55 ± 5	28 ± 5	71 ± 4	21 ± 1	28 ± 4	43 ± 5	4.08

[a] Calculated LogP data using Chemoffice 2005, Chemdraw Ultra 9.0 software. [b] LogP value were calculated based on free base.

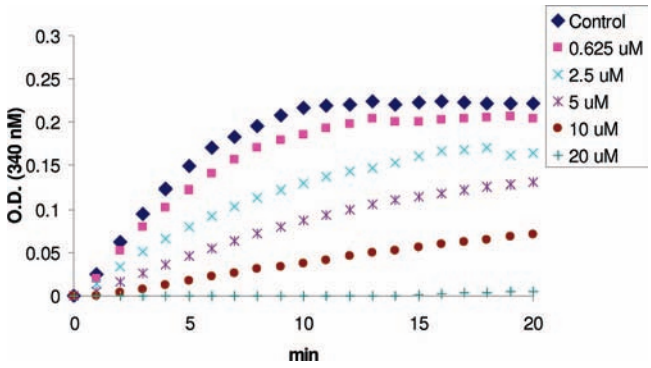


Figure 4. Effect of **8f** on tubulin assembly.

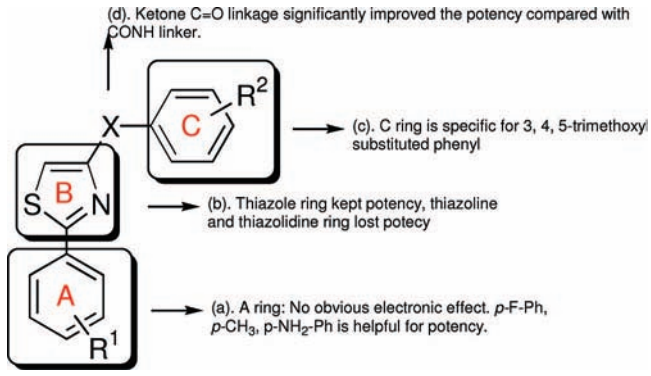


Figure 5. SAR relationship of the SMART molecules.

Mass spectral data was 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).

General Procedure for the Preparation of (2*RS*,4*R*)-2-Aryl-thiazolidine-4-carboxylic (1). 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 solid precipitated out was collected, washed with diethyl ether, and dried to afford according (2*RS*,4*R*)-2-aryl-thiazolidine-4-carboxylic acid **1** with yields of 70−99%. At 0 °C, **1** (5.95 mmol) was dissolved in 1 N NaOH (6 mL) and 1,4-dioxane (15 mL), and 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 vacuum and washed with ethyl acetate (20 mL). The aqueous phase was adjusted to pH = 4 by adding 1 N HCl or 5% $KHSO_4$ and then extracted with ethyl acetate, dried with magnesium sulfate, filtered, and concentrated in vacuum to give corresponding BOC protected acids as white foam-solids, which were used for the next step without further purification.

General Procedure for the Preparation of (2*RS*,4*R*)-2-Aryl-*N*-(3,4,5-trimethoxyphenyl)thiazolidine-4-carboxamide (2a−2b). A mixture of appropriate BOC protected carboxylic acids (0.3−0.5 g), EDCI (1.2 equiv), and HOBT (1.05 equiv) in CH_2Cl_2 (20 mL) was stirred at room temperature for 10 min. To this solution, 3,4,5-trimethoxyaniline (1.05 equiv) and Et_3N (1.2 equiv) were added and stirring continued at room temperature for 6−8 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and sequentially washed with water, satd $NaHCO_3$, brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a crude oil, which were stirred with TFA (0.6−1 mL) in 20 mL CH_2Cl_2 at rt for 1−8 h to cleave the BOC group. The reaction mixture was concentrated, washed with satd $NaHCO_3$, and dried over $MgSO_4$. The solvent was removed to yield a crude solid, **2a**−**2b** were purified by column chromatography. Yield was reported as a two-steps yield.

(2*RS*,4*R*)-2-Phenyl-*N*-(3,4,5-trimethoxyphenyl)thiazolidine-4-carboxamide (2a). Yield: 69.5%; mp 158−159 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.14 (s, 0.8 H), 8.61 (s, 0.2 H), 7.58−7.32 (m, 5 H), 6.90 (s, 1.6 H), 6.71 (s, 0.4H), 5.71 (dd, 0.2 H, J = 9.0 Hz), 5.42 (dd, 0.8 H, J = 11.7 Hz), 4.53 (dt, 0.8 H), 4.19 (m, 0.2 H), 3.87, 3.80 (s, s, 6 H), 3.82, 3.78 (s, s, 3 H), 3.80−3.78 (m, 0.4 H), 3.62−3.42 (m, 1.6 H), 2.96 (t, 0.2 H, J = 9.0 Hz), 2.74 (dd, 0.8 H, J = 11.7 Hz). MS (ESI) m/z 375.1 $[M + H]^+$, 397.1 $[M + Na]^+$. Anal. ($C_{19}H_{22}N_2O_4S$) C, H, N.

(2*RS*,4*R*)-*N*,2-bis(3,4,5-trimethoxyphenyl)thiazolidine-4-carboxamide (2b). Yield: 34.5%; mp 147−149 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.10 (s, 0.7 H), 8.59 (s, 0.3 H), 6.90 (s, 1.4 H), 6.80 (s, 0.6 H), 6.74 (s, 1.4H), 6.71 (s, 0.6 H), 5.66 (br, 0.3 H), 5.35 (d, br, 0.7 H, J = 7.5 Hz), 4.52 (br, 0.7 H), 4.21 (br, 0.3 H), 3.90, 3.87, 3.86, 3.84, 3.82, 3.81, 3.79, 3.78 (all s, 18 H), 3.66−3.61, 3.54−3.38 (m, 1.6 H), 2.98, 2.72 (br, 1 H). MS (ESI) m/z 465.1 $[M + H]^+$, 487.1 $[M + Na]^+$. Anal. ($C_{22}H_{28}N_2O_7S$) C, H, N.

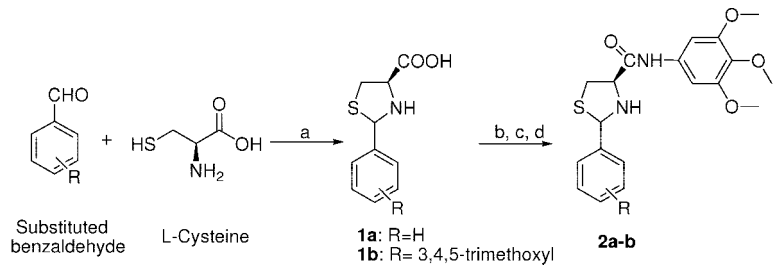
2-(Substituted-phenyl)-4,5-dihydrothiazole-4-carboxylic acids (3). Substituted benzonitrile (40 mmol) was combined with L- or D-cysteine (45 mmol) in 100 mL of 1:1 MeOH/pH6.4 phosphate buffer solution. The reaction was stirred at 40 °C for 3 days. The precipitate was removed by filtration, and MeOH was removed using rotary evaporation. The remaining solution was added 1 M HCl to adjust pH = 4 under 0 °C. The resulting precipitate was extracted into CH_2Cl_2, dried, and concentrated to yield a white to light-yellow solid **3**, which were used directly to the next step without purification.

(4*R*)-2-Phenyl-4,5-dihydrothiazole-4-carboxylic acid (3a). Yield: 58.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.31 (br, 1 H), 7.88−7.85 (m, 2 H), 7.55−7.41 (m, 3 H), 5.38 (t, 1 H, J = 9.6 Hz), 3.75 (dt, 2 H, J = 9.6 Hz, 2.7 Hz). MS (ESI) m/z 162.0 $[M - COOH]^-$.

(4*S*)-2-Phenyl-4,5-dihydrothiazole-4-carboxylic acid (3b). Yield: 53.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89−7.85 (m, 2 H), 7.55−7.41 (m, 3 H), 5.38 (t, 1 H, J = 9.3 Hz), 3.75 (dt, 2 H, J = 9.3 Hz, 2.7 Hz). MS (ESI) m/z 162.0 $[M - COOH]^-$.

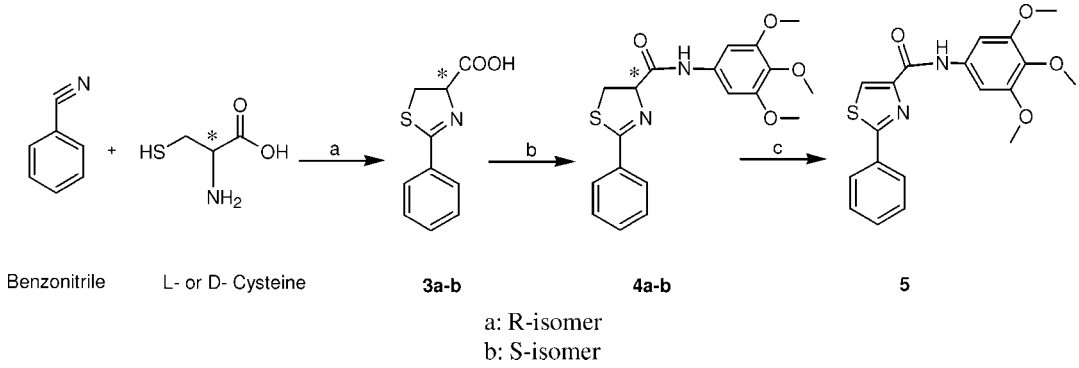
2-Phenyl-*N*-(3,4,5-trimethoxyphenyl)-4,5-dihydrothiazole-4-carboxamide (4a−4b). The compounds were prepared following the same EDCI/HOBt method as **2a**−**2b** described.

Scheme 1[a]



[a] Reagents and conditions: (a) C_2H_5OH, H_2O, rt; (b) Boc_2O, 1 N NaOH, 1, 4-dioxane, H_2O; (c) EDCI, HOBt, TEA, 3,4,5-trimethoxyaniline; (d) TFA, CH_2Cl_2.

Scheme 2[a]



a: R-isomer
b: S-isomer

[a] Reagents and conditions: (a) MeOH/pH = 6.4 phosphate buffer, rt; (b) EDCI, HOBt, TEA, 3,4,5-trimethoxyaniline; (c) $CBrCl_3$, DBU.

(4*R*)-2-Phenyl-*N*-(3,4,5-trimethoxyphenyl)-4,5-dihydrothiazole-4-carboxamide (4a). Yield: 98.7%; mp 121−122 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.98 (s, 1 H), 8.02−7.94, 7.62−7.48 (m, 5 H), 6.93 (s, 2 H), 5.38 (t, 1 H, J = 9.6 Hz), 3.92−3.85 (m, 2 H), 3.87 (s, 6 H), 3.82 (s, 3 H). MS (ESI) m/z 373.1 [M + H]$^+$. Anal. ($C_{19}H_{20}N_2O_4S$) C, H, N.

(4*R*)-2-Phenyl-*N*-(3,4,5-trimethoxyphenyl)-4,5-dihydrothiazole-4-carboxamide (4b). Yield: 70.7%; mp 122−123 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1 H), 7.93−7.90 (m, 2 H), 7.55−7.45 (m, 3 H), 6.88 (s, 2 H), 5.31 (t, 1 H, J = 9.6 Hz), 3.86 (s, 6 H), 3.79 (s, 3 H), 3.83−3.70 (m, 2 H). MS (ESI) m/z 395.1 [M + Na]$^+$, 370.9 [M − 1]$^-$. Anal. ($C_{19}H_{20}N_2O_4S$) C, H, N.

2-Phenyl-*N*-(3,4,5-trimethoxyphenyl)thiazole-4-carboxamide (5). Yield: 89.7%; mp 157−158 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.30 (s, 1 H), 8.20 (s, 1 H), 8.04−8.01 (m, 2 H), 7.53−7.51 (m, 3 H), 7.08 (s, 2 H), 3.92 (s, 6 H), 3.86 (s, 3 H). MS (ESI) m/z 393.1 [M + Na]$^+$. Anal. ($C_{19}H_{18}N_2O_4S$) C, H, N.

2-(Substituted-phenyl)-4,5-dihydrothiazole-4-carboxylic acid methoxymethylamides (6a−6p and 9). General procedure: A mixture of appropriate 2-(substituted-phenyl)-4,5-dihydrothiazole-4-carboxylic acid **3** (5mmol), EDCI (6 mmol), and HOBt (5 mmol) in CH_2Cl_2 (50 mL) was stirred for 10 min. To this solution, NMM (5 mmol) and $HNCH_3OCH_3$ (5 mmol) were added, and stirring continued at room temperature for 6−8 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and sequentially washed with water, satd $NaHCO_3$, brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography.

(*R*)-*N*-Methoxy-*N*-methyl-2-phenyl-4,5-dihydrothiazole-4-carboxamide (6a). Yield: 92.0%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85−7.83 (m, 2 H), 7.48−7.36 (m, 3 H), 5.66 (t, 1 H, J = 9.0 Hz), 3.90 (s, 3 H), 3.88−3.80 (br, 1 H), 3.55−3.47 (dd, 1 H, J = 10.8 Hz, 9.0 Hz), 3.30 (s, 3 H). MS (ESI) m/z 251.0 [M + H]$^+$, 273.0 [M + Na]$^+$.

(*R*)-*N*-Methoxy-*N*-methyl-2-*p*-tolyl-4,5-dihydrothiazole-4-carboxamide (6b). Yield: 55.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, 2 H, J = 7.8 Hz), 7.22 (d, 2 H, J = 7.8 Hz), 5.68 (t, 1 H, J = 8.7 Hz), 3.91 (s, 3 H), 3.80 (t, 1 H, J = 9.3 Hz), 3.55 (t, 1 H, J = 9.3 Hz), 3.30 (s, 3 H), 2.93 (s, 3 H). MS (ESI) m/z 265.0 [M + H]$^+$, 287.0 [M + Na]$^+$.

(*R*)-2-(2-Fluorophenyl)-*N*-methoxy-*N*-methyl-4,5-dihydrothiazole-4-carboxamide (6c). Yield: 39.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dt, 1 H, J = 7.5 Hz, 1.8 Hz), 7.43 (m, 1 H), 7.19−7.09 (m, 2 H), 5.63 (t, 1 H), 3.88 (s, 3 H), 3.83 (br, 1 H), 3.48 (dd, 1 H, J = 11.1 Hz, 9.6 Hz), 3.30 (s, 3 H). MS (ESI) m/z 291.0 [M + Na]$^+$.

(*R*)-2-(3-Fluorophenyl)-*N*-methoxy-*N*-methyl-4,5-dihydrothiazole-4-carboxamide (6d). Yield: 84.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60−7.56 (m, 2 H), 7.38 (dt, 1 H, J = 8.1 Hz, 6.0 Hz), 7.16 (dt, 1 H, J = 8.1 Hz, 2.4 Hz), 5.67 (t, 1 H), 3.90 (s, 3 H), 3.86−3.83 (br, 1 H), 3.52 (dd, 1 H, J = 10.8 Hz, 9.3 Hz), 3.30 (s, 3 H). MS (ESI) m/z 291.0 [M + Na]$^+$.

(*R*)-2-(4-Fluorophenyl)-*N*-methoxy-*N*-methyl-4,5-dihydrothiazole-4-carboxamide (6e). Yield: 66.0%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, 2 H), 7.13 (d, 2 H), 5.63 (t, 1 H), 3.88 (s, 3 H), 3.83 (br, 1 H), 3.46 (dd, 1 H), 3.31 (s, 3 H). MS (ESI) m/z 269.0 [M + H]$^+$.

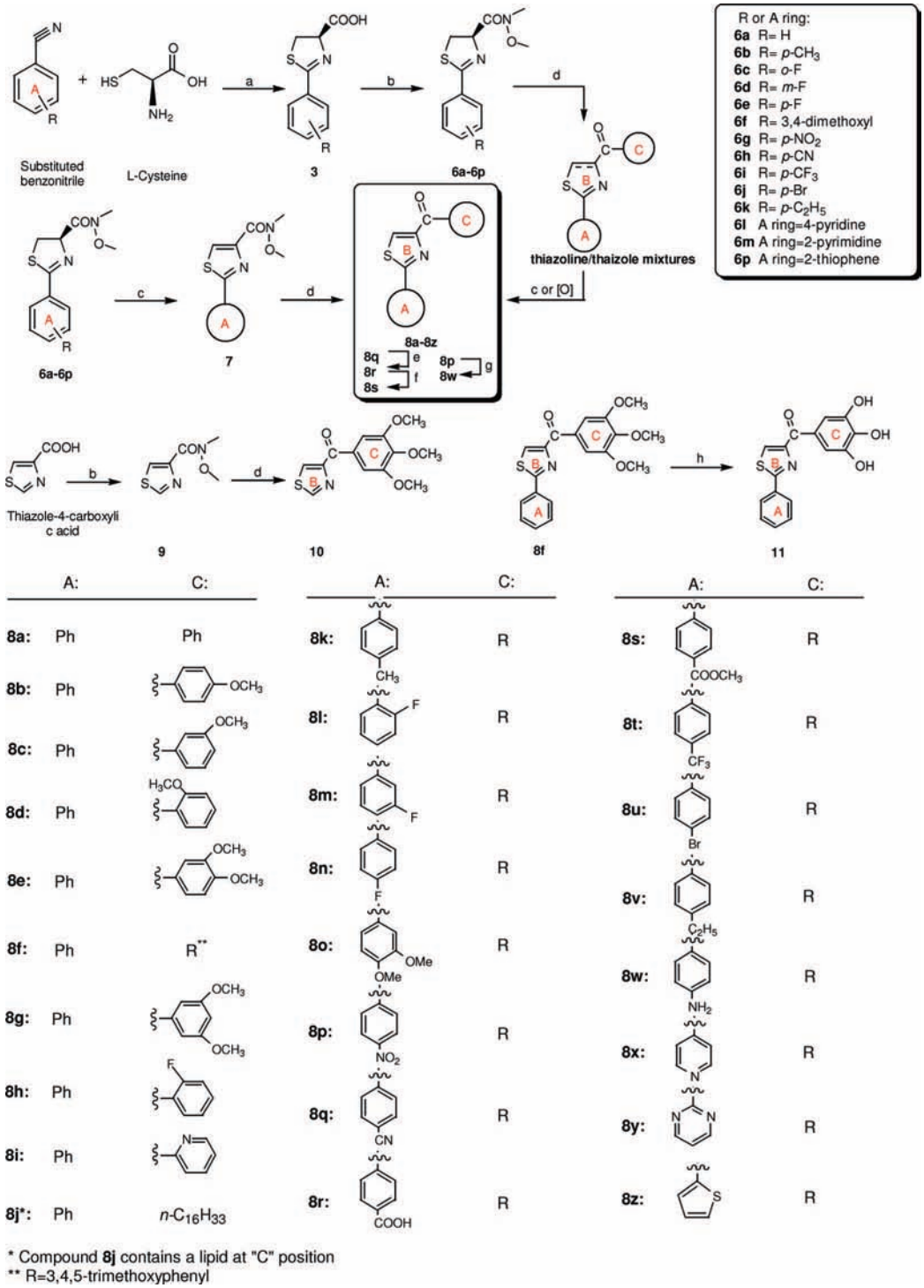
(*R*)-2-(3,4-Dimethoxyphenyl)-*N*-methoxy-*N*-methyl-4,5-dihydrothiazole-4-carboxamide (6f). Yield: 36.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, 1 H), 7.93 (s, 1 H), 7.19−7.09 (d, 1H), 5.41 (t, 1 H), 3.97 (s, 6H), 3.89 (s, 3 H), 3.73 (br, 1 H), 3.39 (dd, 1 H), 3.31 (s, 3 H). MS (ESI) m/z 333.1 [M + Na]$^+$.

(*R*)-*N*-Methoxy-*N*-methyl-2-(4-nitrophenyl)-4,5-dihydrothiazole-4-carboxamide (6g). Yield: 53.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25(d, 2 H, J = 9.0 Hz), 8.01 (d, 2 H, J = 9.0 Hz), 5.73 (t, 1 H), 3.90 (s, 3 H), 3.87 (br, 1 H), 3.59 (dd, 1 H, J = 11.1 Hz, 9.3 Hz), 3.31 (s, 3 H). MS (ESI) m/z 318.1 [M + Na]$^+$.

(*R*)-2-(4-Cyanophenyl)-*N*-methoxy-*N*-methyl-4,5-dihydrothiazole-4-carboxamide (6h). Yield: 26.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94(d, 2 H, J = 8.1 Hz), 7.69 (d, 2 H, J = 8.1 Hz), 5.71 (t, 1 H, J = 9.3 Hz), 3.89 (s, 3 H), 3.87 (br, 1 H), 3.56 (dd, 1 H, J = 10.8 Hz, 9.3 Hz), 3.30 (s, 3 H). MS (ESI) m/z 298.0 [M + Na]$^+$.

(*R*)-*N*-Methoxy-*N*-methyl-2-(4-trifluoromethylphenyl)-4,5-dihydrothiazole-4-carboxamide (6i). Yield: 62.0%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, 2 H, J = 8.1 Hz), 7.65 (d, 2 H, J = 8.1

Scheme 3[a]



[a] Reagents and conditions: (a) MeOH/pH = 6.4 phosphate buffer, rt; (b) EDCI, HOBt, NMM, $HNCH_3OCH_3$; (c) $CBrCl_3$, DBU; (d) ArBr/BuLi or ArMgBr, THF; (e) HCl/HOAc; (f) $MeOH/CH_3COCl$; (g) Fe/HOAc; (h) BBr_3, CH_2Cl_2.

Hz), 5.70 (t, 1 H, J = 9.6 Hz), 3.89 (s, 3 H), 3.85 (br, 1 H), 3.55 (dd, 1 H, J = 10.8 Hz, 9.6 Hz), 3.30 (s, 3 H). MS (ESI) m/z 341.0 $[M + Na]^+$.

(*R*)-2-(4-Bromophenyl)-*N*-methoxy-*N*-methyl-4,5-dihydrothiazole-4-carboxamide (6j). Yield: 20.0%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71, 7.53 (d, d, 4 H, J = 8.4 Hz), 5.63 (t, 1 H, J = 9.6 Hz), 3.88 (s, 3 H), 3.84 (t, 1 H, J = 9.6 Hz), 3.52 (dd, 1 H, J = 10.8 Hz, 9.6 Hz), 3.30 (s, 3 H). MS (ESI) m/z 351.0 $[M + Na]^+$.

(*R*)-*N*-Methoxy-*N*-methyl-2-(4-ethyl)-4,5-dihydrothiazole-4-carboxamide (6k). Yield: 77.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75(d, 2 H, J = 8.4 Hz), 7.21 (d, 2 H, J = 8.4 Hz), 5.64 (t, 1 H), 3.89 (s, 3 H), 3.81 (m, 1 H), 3.48 (dd, 1 H, J = 10.8 Hz, 9.3 Hz), 3.29 (s, 3 H), 2.67 (q, 2 H), 1.24 (t, 3 H). MS (ESI) m/z 301.0 $[M + Na]^+$.

(*R*)-*N*-Methoxy-*N*-methyl-2-(pyridin-4-yl)-4,5-dihydrothiazole-4-carboxamide (6l). Yield: 66.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, 2 H, J = 9.0 Hz), 7.67 (d, 2 H, J = 9.0 Hz), 5.71 (t, 1 H, J = 9.6 Hz), 3.90 (s, 3 H), 3.73 (t, 1 H), 3.55 (dd, 1 H, J = 10.8 Hz, 9.6 Hz), 3.30 (s, 3 H). MS (ESI) m/z 252.1 $[M + H]^+$, 274.0 $[M + Na]^+$.

(*R*)-*N*-Methoxy-*N*-methyl-2-(pyrimidin-2-yl)-4,5-dihydrothiazole-4-carboxamide (6m). Yield: 32.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.88 (d, 2 H, J = 4.8 Hz), 7.38 (t, 1 H, J = 4.8 Hz), 5.83

(t, 1 H, $J = 9.0$ Hz), 3.87 (s, 3 H), 3.56 (dd, 2 H, $J = 9.0$ Hz), 3.30 (s, 3 H). MS (ESI) m/z 275.0 $[M + Na]^+$.

(*R*)-*N*-Methoxy-*N*-methyl-2-(thiophen-2-yl)-4,5-dihydrothiazole-4-carboxamide (6p). Yield: 58.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (br, 1 H), 7.49 (d, 1 H, $J = 4.8$ Hz), 7.09 (dd, 1 H, $J = 3.6$ Hz, 4.8 Hz), 5.64 (t, 1 H, $J = 9.0$ Hz), 3.90 (s, 3 H), 3.85 (br, 1 H), 3.57 (dd, 1 H, $J = 9.9$, 9.0 Hz), 3.29 (s, 3 H). MS (ESI) m/z 279.0 $[M + Na]^+$.

***N*-Methoxy-*N*-methylthiazole-4-carboxamide (9).** Yield: 58.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.82 (d, 1 H, $J = 2.1$ Hz), 8.10 (d, 1 H, $J = 2.1$ Hz), 3.79 (s, 3 H), 3.45 (s, 3 H). MS (ESI) m/z 194.9 $[M + Na]^+$.

2-(Substituted-phenyl)-thiazole-4-carboxylic Acid Methoxymethylamide (7). General procedure: A solution of **6a**−**6p** (1 equiv) in CH_2Cl_2 was cooled to 0 °C, and distilled DBU (2 equiv) was added. Bromotrichloromethane (1.7 equiv) was then introduced dropwise via syringe over 10 min. The reaction mixtures were allowed to warm to room temperature and stirred overnight. Upon washing with satd aqueous NH_4Cl (2 × 50 mL), the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried on $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography as needed providing compounds **7**.

2-Phenyl-thiazole-4-carboxylic Acid Methoxymethylamide. Yield: 73.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (s, 1 H), 7.99−7.96 (m, 2 H), 7.47−7.44 (m, 3 H), 3.88 (s, 3 H), 3.49 (s, 3 H). MS (ESI) m/z 271.0 $[M + Na]^+$.

[2-(Substituted-phenyl)-thiazol-4-yl]-(3,4,5-trimethoxy-phenyl)-methanone (8a−8z and 10). Method 1: To a solution of *n*-BuLi (1.6M, 0.713 mL) in 8 mL of THF was added a solution of 3,4,5-trimethoxybromobenzene (1.09 mmol) in 3 mL of THF under −78 °C. The mixture was stirred for 2 h, and a solution of amides **6** or **7** (1.14 mmol) in 3 mL of THF was charged. The mixture was allowed to warm to room temperature and stirred for overnight. The reaction mixture was quenched with satd NH_4Cl, extracted with ethyl ether, dried with $MgSO_4$, and exposed in air atmosphere for overnight. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **8a**−**8z**. Method 2: To a solution of corresponding Grignard reagents (0.5M, 3 mL) in 2 mL THF was charged a solution of amides **6** or **7** (1 mmol) in 3 mL of THF at 0 °C. The mixtures were stirred for 30 min to 2 h until amides disappeared on TLC plates. The reaction mixture was quenched with satd NH_4Cl, extracted with ethyl ether, dried with $MgSO_4$, and set in air atmosphere overnight to yield **6** as starting material. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound **8a**−**8z**. Accordingly, hydrochloride salt was prepared as following: At 0 °C, to a solution of 10 mL of HCl in ethyl ether (2 M) solution was added **8i**, **8x**, or **8w** (100 mg) in 5 mL of CH_2Cl_2 (5 mL) and stirred overnight. The hydrochloride precipitate was filtered and washed with ethyl ether. Dying under high vacuum yielded the corresponding salts.

Phenyl (2-Phenylthiazol-4-yl)-methanone (8a). Yield: 76.3%; mp 65−66 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32−8.29 (m, 2 H), 8.24 (s, 1 H), 8.04−8.00 (m, 2 H), 7.64−7.52 (m, 3 H), 7.50−7.46 (m, 3 H). MS (ESI) m/z 288.0 $[M + Na]^+$. Anal. ($C_{16}H_{11}NOS$) C, H, N.

(4-Methoxyphenyl)(2-phenylthiazol-4-yl)-methanone (8b). Yield: 74.8%; mp 105−106 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (d, 2 H), 8.22 (s, 1 H), 8.02 (dd, 2 H), 7.47 (m, 3 H), 7.01 (d, 2 H), 3.80 (s, 3 H). MS (ESI) m/z 318.1 $[M + Na]^+$. Anal. ($C_{17}H_{13}NO_2S$) C, H, N.

(3-Methoxyphenyl)(2-phenylthiazol-4-yl)-methanone (8c). Yield: 58.8%; mp 43−44 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (s, 1 H), 8.05−8.01 (m, 2 H), 7.93 (d, 1 H), 7.84 (m, 1 H), 7.49−7.40 (m, 4 H), 7.16−7.15 (m, 1 H), 3.89 (s, 3 H). MS (ESI) m/z 318.1 $[M + Na]^+$. Anal. ($C_{17}H_{13}NO_2S$) C, H, N.

(2-Methoxyphenyl)(2-phenylthiazol-4-yl)-methanone (8d). Yield: 57.4%; colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1 H), 7.98−7.95 (m, 2 H), 7.57−7.47 (m, 2 H), 7.47−7.42 (m, 3 H), 7.08−7.01 (m, 2 H), 3.78 (s, 3 H). MS (ESI) m/z 318.1 $[M + Na]^+$. Anal. ($C_{17}H_{13}NO_2S$) C, H, N.

(3,4-Dimethoxyphenyl)(2-phenylthiazol-4-yl)-methanone (8e). Yield: 15.3%; mp 89−91 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (s, 1 H), 8.22 (dd, 1 H, $J = 8.5$ Hz, 2.0 Hz), 8.04−8.02 (m, 2 H), 7.99 (d, 1 H, $J = 2.0$ Hz), 7.49−7.47 (m, 3 H), 6.98 (d, 1 H, $J = 8.5$ Hz), 3.99 (s, 6 H). MS (ESI) m/z 348.0 $[M + Na]^+$. Anal. ($C_{18}H_{15}NO_3S$) C, H, N.

(2-Phenyl-thiazol-4-yl)-(3,4,5-trimethoxy-phenyl)-methanone (8f). Yield: 27.3%; mp 133−135 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (s, 1 H), 8.03 (q, 2 H), 7.80 (s, 2 H), 7.49−7.47 (m, 3 H), 3.96 (s, 6 H), 3.97 (s, 3 H). MS (ESI) m/z 378.1 $[M + Na]^+$. Anal. ($C_{19}H_{17}NO_4S$) C, H, N.

(3,5-Dimethoxyphenyl)(2-phenylthiazol-4-yl)-methanone (8g). Yield: 41.5%; mp 84−85 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (s, 1 H), 8.04−8.01 (m, 2 H), 7.99 (d, 2 H, $J = 2.4$ Hz), 7.49−7.43 (m, 3 H), 6.72 (t, 1 H, $J = 2.4$ Hz), 3.87 (s, 6 H). MS (ESI) m/z 348.3 $[M + Na]^+$. Anal. ($C_{18}H_{15}NO_3S$) C, H, N.

(2-Fluorophenyl)(2-phenylthiazol-4-yl)-methanone (8h). Yield: 66.4%; mp 77−79 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48−8.41 (m, 2 H), 8.28 (s, 2 H), 8.04−7.98 (m, 2 H), 7.50−7.46 (m, 3 H), 7.26−7.16 (m, 2 H). MS (ESI) m/z 306.0 $[M + Na]^+$, 283.9 $[M - H]^-$. Anal. ($C_{16}H_{10}FNOS$) C, H, N.

(2-Phenylthiazol-4-yl)-(pyridin-2-yl)-methanone (8i). Yield: 20.7%; mp 95−97 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.01 (s, 1 H), 8.77 (d, 1 H, $J = 4.8$ Hz), 8.28 (d, 1 H, $J = 7.8$ Hz), 8.08−8.05 (m, 2 H), 7.92 (dt, 1 H, $J = 7.8$ Hz, 1.2 Hz), 7.52 (ddd, 1 H, $J = 7.8$ Hz, 4.8 Hz, 1.2 Hz), 7.48−7.46 (m, 3 H). (**8i·HCl**): Yield: 70.6%; mp 105−107 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.03 (s, 1 H), 8.79 (d, 1 H, $J = 4.8$ Hz), 8.10 (br, 1 H), 8.08 (br, 1 H), 8.03−8.00 (m, 2 H), 7.73−7.69 (m, 1 H), 7.56−7.54 (m, 3 H). MS (ESI) m/z 267.0 $[M + H]^+$. Anal. ($C_{15}H_{10}N_2OS$, $C_{15}H_{10}N_2OS\cdot HCl$) C, H, N.

1-(2-Phenylthiazol-4-yl)-heptadecan-1-one (8j). Yield: 66.4%; mp 63−64 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1 H), 8.02−7.99 (m, 2 H), 7.49−7.47 (m, 3 H), 3.16 (t, 2 H, $J = 7.5$ Hz), 1.82−1.72 (m, 2 H), 1.26 (s, 26 H), 0.88 (t, 3 H, $J = 6.9$ Hz). MS (ESI) m/z 414.4 $[M + H]^+$. Anal. ($C_{26}H_{39}NOS$) C, H, N.

(2-*p*-Tolylthiazol-4-yl)-(3,4,5-trimethoxyphenyl)-methanone (8k). Yield: 53.2%; mp 116−119 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1 H), 7.91 (d, 2 H, $J = 8.1$ Hz), 7.80 (s, 2 H), 7.28 (d, 2 H, J= 8.1 Hz), 3.96 (s, 3 H), 3.95 (s, 6 H). MS (ESI) m/z 392.1 $[M + Na]^+$. Anal. ($C_{20}H_{19}NO_4S$) C, H, N.

[2-(2-Fluorophenyl)-thiazol-4-yl]-(3,4,5-trimethoxyphenyl)-methanone (8l). Yield: 39.6%; mp 90−102 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (s, 1 H), 8.33 (dt, 1 H, $J = 1.5$ Hz, 8.0 Hz), 7.78 (s, 2 H), 7.49−7.44 (m, 1 H), 7.30−7.23 (m, 2 H), 3.97 (s, 3 H), 3.95 (s, 6 H). MS (ESI) m/z 396.1 $[M + Na]^+$. Anal. ($C_{19}H_{16}FNO_4S$) C, H, N.

[2-(3-Fluorophenyl)-thiazol-4-yl](3,4,5-trimethoxyphenyl)-methanone (8m). Yield: 14.1%; mp 122−124 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1 H), 7.79 (s, 2 H), 7.76−7.74 (m, 2 H), 7.45 (dt, 1 H, $J = 6.0$ Hz, 8.4 Hz), 7.18 (dt, 1 H, $J = 1.8$ Hz, 8.4 Hz), 3.97 (s, 3 H), 3.96 (s, 6 H). MS (ESI) m/z 396.1 $[M + Na]^+$. Anal. ($C_{19}H_{16}FNO_4S$) C, H, N.

[2-(4-Fluorophenyl)-thiazol-4-yl]-(3,4,5-trimethoxyphenyl)-methanone (8n). Yield: 40.2%; mp 153−155 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (s, 1 H), 8.04−8.00 (dd, 2 H, $J = 8.4$ Hz, 5.7 Hz), 7.75 (s, 2 H), 7.21−7.15 (t, 3 H, $J = 8.4$ Hz), 3.97 (s, 3 H), 3.95 (s, 6 H). MS (ESI) m/z 396.1 $[M + Na]^+$. Anal. ($C_{19}H_{16}FNO_4S$) C, H, N.

[2-(3,4-Dimethoxyphenyl)-thiazol-4-yl]-(3,4,5-trimethoxyphenyl)-methanone (8o). Yield: 46.6%; mp 145−147 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (s, 1 H), 7.76 (s, 2 H), 7.58−7.54 (m, 2 H), 6.94 (d, 2 H, $J = 8.1$ Hz), 3.96 (s, 6 H), 3.95 (s, s, 9H). MS (ESI) m/z 438.1 $[M + Na]^+$. Anal. ($C_{21}H_{21}NO_6S\cdot 1/4H_2O$) C, H, N.

[2-(4-Nitrophenyl)-thiazol-4-yl]-(3,4,5-trimethoxyphenyl)-methanone (8p). Yield: 46.4%; mp 199−200 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (d, 2 H, $J = 8.7$ Hz), 8.34 (s, 1 H), 8.20 (d, 2 H, J

= 8.7 Hz), 7.73 (s, 2 H), 3.98 (s, 3 H), 3.95 (s, 6 H). MS (ESI) *m/z* 423.1 $[M + Na]^+$. Anal. ($C_{19}H_{16}N_2O_6S$) C, H, N.

4-[4-(3,4,5-Trimethoxybenzoyl)-thiazol-2-yl]-benzonitrile (8q). Yield: 45.9%; mp 181−182 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1 H), 8.13 (d, 2 H, J = 8.4 Hz), 7.78 (d, 2 H, J = 8.4 Hz), 7.72 (s, 2 H), 3.97 (s, 3 H), 3.94 (s, 6 H). MS (ESI) *m/z* 403.1 $[M + Na]^+$. Anal. ($C_{20}H_{16}N_2O_4S$) C, H, N.

4-[4-(3,4,5-Trimethoxybenzoyl)-thiazol-2-yl]-benzoic acid (8r). Yield: 61.9%; mp > 220 °C (dec). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (s, 1 H), 8.00 (d, d, 4 H), 7.65 (s, 2 H), 3.88 (s, 6 H), 3.80 (s, 3 H). MS (ESI) *m/z* 397.9 $[M - H]^-$, 353.9 $[M - COOH]^-$. Anal. ($C_{20}H_{17}NO_6S$) C, H, N.

Methyl-4-[4-(3,4,5-trimethoxybenzoyl)-thiazol-2-yl]-benzoate (8s). Yield: 72.5%; mp 172−174 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1 H), 8.12 (dd, 4 H, J = 8.4 Hz), 7.78 (s, 2 H), 3.97 (s, 3 H), 3.96 (s, 3H), 3.95 (s, 6 H). MS (ESI) *m/z* 436.1 $[M + Na]^+$. Anal. ($C_{21}H_{19}NO_6S$) C, H, N.

(2-(4-(Trifluoromethyl)-phenyl)-thiazol-4-yl)(3,4,5-trimethoxyphenyl)-methanone (8t). Yield: 45.5%; mp 144−145 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1 H), 8.14, 7.65 (d, d, 4 H, J = 8.1 Hz), 7.76 (s, 2 H), 3.97 (s, 3 H), 3.95 (s, 6 H). MS (ESI) *m/z* 446.1 $[M + Na]^+$. Anal. ($C_{20}H_{16}F_3NO_4S$) C, H, N.

[2-(4-Bromophenyl)-thiazol-4-yl]-(3,4,5-trimethoxyphenyl)-methanone (8u). Yield: 51.8%; mp 149−150 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (s, 1 H), 7.89, 7.62 (d, d, 4 H, J = 8.1 Hz), 7.75 (s, 2 H), 3.97 (s, 3 H), 3.94 (s, 6 H). MS (ESI) *m/z* 456.0, 458.0 $[M + Na]^+$. Anal. ($C_{19}H_{16}BrNO_4S$) C, H, N.

[2-(4-Ethyl-phenyl)-thiazol-4-yl]-(3,4,5-trimethoxy-phenyl)-methanone (8v). Yield: 40.0%; mp 86−87 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1 H), 7.93, 7.31 (d, d, 4 H, J = 8.4 Hz), 7.81 (s, 2 H), 3.97 (s, 3 H), 3.95 (s, 6 H). MS (ESI) *m/z* 406.1 $[M + Na]^+$. Anal. ($C_{21}H_{21}NO_4S$) C, H, N.

[2-(4-Amino-phenyl)-thiazol-4-yl]-(3,4,5-trimethoxy-phenyl)-methanone (8w). Yield: 61.8%; mp 177−179 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (s, 1 H), 7.82, 7.65 (d, d, 4 H, J = 8.4 Hz), 7.78 (s, 2 H), 3.96 (s, 3 H), 3.94 (s, 6 H). (**8w·HCl**): Yield: 50.1%; mp 166−169 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.49 (s, 1 H), 7.84, 6.94 (d, d, 4 H, J = 8.4 Hz), 7.62 (s, 2 H), 3.86 (s, 3 H), 3.79 (s, 6 H). MS (ESI) *m/z* 393.1 $[M + Na]^+$. Anal. ($C_{19}H_{18}N_2O_4S$, $C_{19}H_{18}N_2O_4S$·HCl) C, H, N.

[2-(Pyridin-4-yl)-thiazol-4-yl]-(3,4,5-trimethoxyphenyl)-methanone (8x). Yield: 29.3%; mp 178−180 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.77 (dd, 2 H, J = 6.0 Hz, 1.5 Hz), 8.40 (s, 1 H), 7.87 (dd, 2 H, J = 6.0 Hz, 1.8 Hz), 7.75 (s, 2 H), 3.98 (s, 3 H), 3.95 (s, 6 H). (**8x·HCl**): Yield: 92.7%; mp 182−184 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.85 (br, 2 H), 8.52 (s, 1 H), 8.22 (br, 2 H), 7.66 (s, 2 H), 3.98 (s, 3 H), 3.94 (s, 6 H). MS (ESI) *m/z* 379.1 $[M + Na]^+$. Anal. ($C_{18}H_{16}N_2O_4S$, $C_{18}H_{16}N_2O_4S$·HCl) C, H, N.

[2-(Pyrimidin-2-yl)-thiazol-4-yl]-(3,4,5-trimethoxyphenyl)-methanone (8y). Yield: 51.9%; mp 190−191 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.88 (d, 2 H, J = 4.8 Hz), 8.44 (s, 1 H), 7.73 (s, 2 H), 7.37 (t, 1 H, J = 4.8 Hz), 3.95 (s, 3 H), 3.94 (s, 6 H). MS (ESI) *m/z* 380.1 $[M + Na]^+$. Anal. ($C_{17}H_{15}N_3O_4S$) C, H, N.

[2-(Thiophen-2-yl)-thiazol-4-yl]-(3,4,5-trimethoxyphenyl)-methanone (8z). Yield: 30.5%; mp 111−113 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1 H), 7.90 (s, 2 H), 7.58 (dd, 1 H, J = 3.6, 0.9 Hz), 7.46 (dd, 1 H, J = 5.4, 0.9 Hz), 7.12 (dd, 1 H, J = 5.4, 3.6 Hz), 3.98 (s, 6 H), 3.97 (s, 3 H). MS (ESI) *m/z* 384.1 $[M + Na]^+$. Anal. ($C_{17}H_{15}NO_4S_2$) C, H, N.

Thiazol-4-yl-(3,4,5-trimethoxy-phenyl)-methanone (10). Yield: 49.4%; mp 106−108 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.92 (d, 1 H, J = 2.1 Hz), 8.34 (d, 1 H, J = 2.1 Hz), 7.61 (s, 2 H), 3.94 (s, 3 H), 3.93 (s, 6 H). MS (ESI) *m/z* 302.0 $[M + Na]^+$. Anal. ($C_{13}H_{13}NO_4S$) C, H, N.

(2-Phenyl-thiazol-4-yl)-(3,4,5-trihydroxy-phenyl)-methanone (11). To a solution of **8f** (123 mg, 0.35 mmol) in 5 mL anhyd CH_2Cl_2 was added BBr_3 (1 M solution in CH_2Cl_2, 1.75 mL, 5 mmol) under −78 °C. The mixture was stirred for 2 h and a solution of amide **7** (1.14 mmol) in 3 mL of THF was charged. The mixture was allowed to warm to room temperature slowly and stirred overnight. The reaction mixture was quenched with satd NH_4Cl, extracted with ethyl acetate, and dried with $MgSO_4$. The solvent was removed under reduced pressure to yield a crude product, which was purified by column chromatography to obtain pure compound as red crystalline solid. Yield: 50.9%; mp 175−176 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.44 (d, 1 H), 8.07−8.04 (m, 2 H), 7.57−7.55 (m, 3 H), 7.33 (s, 2 H). MS (ESI) *m/z* 336.1 $[M + Na]^+$. Anal. ($C_{16}H_{11}NO_4S$) C, H, N.

X-ray Crystallography Structure Determination. X-ray crystallographic data for **8f** were collected from a single crystal mounted with paratone oil on a nylon cryoloop. Data were collected at 100 K on a Bruker Proteum CCD area detector, controlled by Proteum2 software,[26] using a rotating-anode generator and Osmic mirrors to generate Cu radiation (λ = 1.54178 Å). The data were reduced using SAINT,[27] with an absorption correction applied using SADABS[28] based on redundant reflections; this correction included a spherical component. The structure was solved using direct methods (SHELXS^{x4}), which revealed all of the heavy atoms. Structure refinement with SHELXL[29] was carried out using full-matrix methods based on F^2 and proceeded smoothly. Hydrogen atoms were added to the structural model assuming ideal C−H distances and isotropic ADPs constrained to be similar to that of the bonded carbon atom. In the final model, anisotropic ADPs were refined for all heavy atoms and isotropic ADPs for chemically similar hydrogens (e.g., methyl H) were constrained to be identical. The final refinement parameters are: wR2 = 0.084 for 228 parameters and 3066 independent observations, R1 = 0.031, S (goodness-of-fit) = 1.057. The final structure has been submitted to the Cambridge Crystallographic Data Center for deposition.

Biology.

Cell Culture and Cytotoxicity Assay of Melanoma. We examined the antiproliferative activity of the ATCAA and SMART analogues in one human melanoma cell line (A375) 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). Human dermal fibroblast cells were purchased from Cascade Biologics, Inc., Portland, OR. All cell lines were cultured in DMEM (Cellgro Mediatech, Inc., Herndon, VA), supplemented with 5% FBS (Cellgro Mediatech), 1% antibiotic/antimycotic mixture (Sigma-Aldrich, Inc., St. Louis, MO), and bovine insulin (5 μg/mL; Sigma-Aldrich). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. 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. IC_{50} (i.e., concentration which inhibited cell growth by 50% of no treatment controls) values were obtained by nonlinear regression analysis with GraphPad Prism (GraphPad Software, San Diego,CA).

Cell Culture and Cytotoxicity Assay of Prostate Cancer. We examined the antiproliferative activity of the ATCAA and SMART analogues in four human prostate cancer cell lines (LNCaP, DU 145, PC-3, and PPC-1). LNCaP, PC-3, and DU 145 cells were purchased from ATCC (American Type Culture Collection, Manassas,VA). Dr. Mitchell Steiner at University of Tennessee Health Science Center kindly provided PPC-1cells. All prostate cancer cell lines were cultured in RPMI 1640 (Cellgro Mediatech, Inc., Herndon, VA), supplemented with 10% FBS (Cellgro Mediatech). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Then 1000−5000 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 3−5 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 IC_{50} (concentration that inhibited cell growth by

50% of untreated control) values were obtained by nonlinear regression analysis using WinNonlin (Pharsight Corporation, Mountain View, CA).

In Vitro Microtubule Polymerization Assay. Bovine brain tubulin (0.4 mg) (Cytoskeleton, Denver, CO) was mixed with various concentrations (0.625−20 μM) of test compound and incubated in 120 μL of general tubulin buffer (80 mM PIPES, 2.0 mM $MgCl_2$, 0.5 mM EGTA, pH 6.9, and 1 mM GTP). The absorbance of wavelength at 340 nm was monitored every 60 s for 20 min by the SYNERGY 4 microplate reader (Bio-Tek Instruments, Winooski, VT). The spectrophotometer was set at 37 °C for tubulin polymerization. The IC_{50} value was defined as the concentration which can inhibit 50% of microtubule polymerization.

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Supporting Information Available: Additional spectral data for compounds, crystallographic information file of compound **8f**, and NCI data for compound ATCAA-1. This material is available free of charge via the Internet at http://pubs.acs.org.

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