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Synthesis and antiproliferative activity of thiazolidine analogs for melanoma

Wei Li,[a] Yan Lu,[a] Zhao Wang,[a] James T. Dalton[b] and Duane D. Miller[a,*]

[a]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN 38163, USA*

[b]*Division of Pharmaceutics, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*

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Abstract—We have previously described 2-aryl-thiazolidine-4-carboxylic acid amides as a novel class of antiproliferative agents for prostate cancer. Screening these compounds with melanoma cell lines revealed that several of them have potent antiproliferative activity and selectivity against melanoma. To further improve the potency and selectivity, we synthesized a new series of analogs and tested them in two melanoma cell lines and fibroblast cells (negative controls). Comparison of anticancer effects of these compounds with a standard chemotherapeutic agent, sorafenib, showed that they are very effective in killing melanoma cells with low micromolar to nanomolar antiproliferative activity and provide us a new lead for developing potential drugs for melanoma.

Melanoma is the most aggressive form of skin cancer and is the fastest growing cancer in the United States.[1,2] Early stage melanoma can be cured surgically. However, melanoma metastasizing to major organs (stage IV) is virtually incurable.[2] Patients with advanced melanoma have a median survival time of less than a year, and the estimated 5-year survival rate is less than 15%.[2,3] Dacarbazine (DTIC) is the only FDA-approved drug to treat advanced melanoma, but complete remission after DTIC treatment rarely exceeds 5% in patients.[4,5] Although additional adjuvant treatment such as the use of high-dose interferon alpha-2b (IFN-α2b) for patients at high risk of recurrence of melanoma has also been approved by FDA, extensive clinical trials have not detected a survival advantage with the addition of IFN to DTIC.[6–9] Metastatic melanoma is invariably resistant to existing agents including triazenes (DTIC or Temozolomide),[10,11] nitrosoureas (BCNU, CCNU, or Fotemustine),[12,13] as well as combination therapies such as cisplatin and etoposide.[14–16]

Several extensive clinical trials have been conducted in recent years with a variety of cancer drugs or combination of cancer drugs, including DTIC combined with cisplatin, vinblastine, or carmustine,[17,18] but they all have failed to demonstrate a clear effect against advanced melanoma.[4,6,16,19] Therefore, DTIC remains the gold standard for advanced melanoma despite its very limited efficacy.[20,21] With the rapidly rising incidence reported for melanoma in the United States, clearly there is an urgent need to develop more effective therapeutic agents to combat advanced melanoma.

Recently, novel classes of lipid compounds have been synthesized and have shown strong activity toward prostate cancer cells.[22,23] Although this class of compounds was initially designed as inhibitors of lysophosphatidic acid (LPA) receptors, we discovered that they did not bind to LPA. They are unlikely to be DNA alkylating agents such as DTIC. Encouraged by the results with prostate cancer, we decided to test a library of such compounds against metastatic melanoma in vitro. The initial results provided us with three classes of highly potent lead compounds for metastatic melanoma. The most potent lead compound has an IC_{50} value in the submicromole range with 10-fold selectivity against cancer cells.[24] To further improve potency and selectivity, we performed extensive synthesis and biological testing of additional compounds in this series. Here we report the synthesis and in vitro antiproliferative activity of these new compounds against two human

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* Corresponding author. Tel.: +1 901 448 6026; fax: +1 901 448 3446; e-mail: dmiller@utmem.edu

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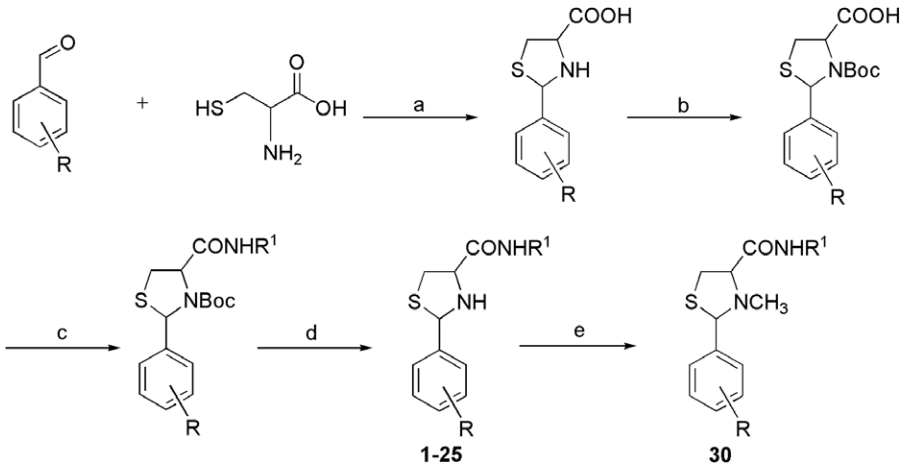


Scheme 1. Reagents: (a) C_2H_5OH, H_2O (70–99%); (b) Boc_2O, 1 N NaOH, 1,4-dioxane, H_2O (89–97%); (c) EDCI, HOBt, Et_3N, amine, CH_2Cl_2; (d) TFA (42–68% two steps); (e) HCHO, $NaBH_3CN$, HOAc (67%).

melanoma cell lines and fibroblast cells to determine their selectivity.

The general synthesis of 2-aryl-thiazolidine-4-carboxylic acid amides is shown in Scheme 1.

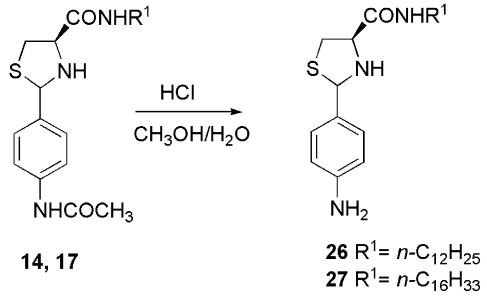
L- or D-Cysteine was reacted with appropriate benzaldehydes in ethanol and water at ambient temperature to give cyclized 2-aryl-thiazolidine-4-carboxylic acid, which was converted to the corresponding Boc derivatives. Reaction of Boc-protected carboxylic acids with different amines using EDC/HOBt gave corresponding amides, which were treated with TFA to form the target compounds **1**–**25**.[22] Reductive alkylation with formaldehyde and sodium cyanoborohydride of the amino group in compound **17** gave methylation derivative **30**.[25] Dimer **31** was obtained by intramolecular condensation of 2-aryl-thiazolidine-4-carboxylic acid with EDC/HOBt.

Derivatives **26** and **27** with a 4-amino-phenyl group were synthesized by deacetylation of compounds **14** and **17**, which was accomplished by acid hydrolysis in methanol (Scheme 2).

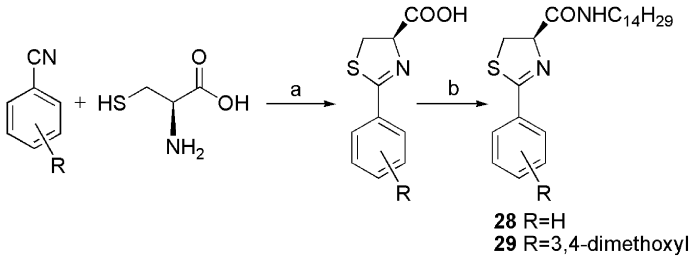
L-Cysteine and appropriate benzonitriles were dissolved in a 1:1 (v/v) mixture of phosphate buffer (pH 6.4) and methanol and stirred at 50 °C to give cyclized 2-aryl-4,5-dihydro-thiazole-4-carboxylic acid, which was reacted with tetradecylamine using EDC/HOBt to give corresponding compounds **28** and **29** as shown in Scheme 3.[26]

We characterized each compound with NMR, mass spectroscopy, and elemental analysis.

We examined the antiproliferative activity of these newly synthesized compounds in two human melanoma cell lines (SK-MEL-188 and WM-164) and in a fibroblast cell line. We used activity on fibroblast cells as a control to determine the selectivity of these compounds against melanoma. Standard sulforhodamine B (SRB) 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., concen-

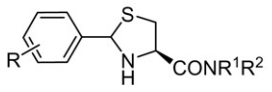
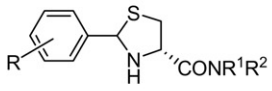
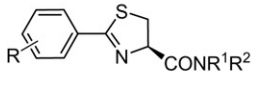
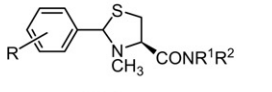
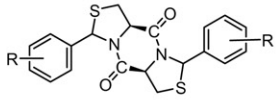


Scheme 2.



Scheme 3. Reagents: (a) MeOH/pH 6.4 phosphate buffer (58–75%); (b) EDCI, HOBt, Et_3N, n-$C_{14}H_{29}NH_2$, CH_2Cl_2 (58–87%).

Table 1. Antiproliferative activity of thiazolidine analogs and their comparison with that of sorafenib and DTIC (ND, not detected)

Structure	Compound	R	R^1	R^2	IC_{50} ± SEM (μM)		
					SK-MEL-188	WM-164	Fibroblast
	**1**	3,4,5-Trimethoxyl	n-C_8H_{17}	H	17.1 ± 0.6	19.6 ± 0.9	20.8 ± 10.4
	2	3,4,5-Trimethoxyl	n-$C_{10}H_{21}$	H	14.5 ± 2.8	2.1 ± 0.4	6.7 ± 3.9
	3	3,4,5-Trimethoxyl	n-$C_{12}H_{25}$	H	2.1 ± 0.4	2.4 ± 0.4	2.4 ± 1.2
	4	3,4,5-Trimethoxyl	n-$C_{14}H_{29}$	H	2.0 ± 0.5	1.6 ± 0.4	2.6 ± 0.4
	5	3,4,5-Trimethoxyl	n-$C_{16}H_{33}$	H	1.8 ± 0.2	0.7 ± 0.1	2.4 ± 0.4
	6	3,4-Dimethoxyl	n-$C_{10}H_{21}$	H	11.9 ± 5.6	6.1 ± 2.5	6.3 ± 1.1
	7	3,4-Dimethoxyl	n-$C_{12}H_{25}$	H	2.9 ± 0.9	1.6 ± 0.5	4.5 ± 1.9
	8	3,4-Dimethoxyl	n-$C_{14}H_{29}$	H	1.5 ± 0.5	0.8 ± 0.3	2.8 ± 1.1
	9	3,4-Dimethoxyl	n-$C_{16}H_{33}$	H	1.5 ± 0.6	0.5 ± 0.2	2.1 ± 0.8
	10	3,4-OCH_2O–	n-$C_{10}H_{21}$	H	6.6 ± 0.5	4.5 ± 0.1	8.2 ± 3.1
	11	3,4-OCH_2O–	n-$C_{12}H_{25}$	H	3.5 ± 0.1	2.5 ± 0.1	5.2 ± 1.2
	12	3,4-OCH_2O–	n-$C_{14}H_{29}$	H	1.6 ± 0.1	1.0 ± 0.1	4.2 ± 0.5
	13	3,4-OCH_2O–	n-$C_{16}H_{33}$	H	1.6 ± 0.1	1.8 ± 0.1	5.7 ± 1.8
	14	$NHCOCH_3$	n-$C_{12}H_{25}$	H	2.4 ± 0.1	1.2 ± 0.1	3.5 ± 0.4
	15	$NHCOCH_3$	n-$C_{14}H_{29}$	H	2.3 ± 01	0.6 ± 0.1	3.6 ± 0.8
	16	$NHCOCH_3$	n-$C_{15}H_{31}$	H	1.6 ± 0.1	1.0 ± 0.1	14.3 ± 2.1
	17	$NHCOCH_3$	n-$C_{16}H_{33}$	H	2.1 ± 0.2	0.6 ± 0.1	19.1 ± 7.7
	18	$NHCOCH_3$	n-$C_{17}H_{35}$	H	8.5 ± 0.1	2.4 ± 0.1	35.8 ± 5.0
	19	$NHCOCH_3$	n-$C_{18}H_{37}$	H	22.3 ± 2.8	11.6 ± 0.6	>60
	20Z	$NHCOCH_3$	(*Z*)-Octadec-8-enyl	H	1.4 ± 0.1	1.0 ± 0.1	10.6 ± 0.9
	20E	$NHCOCH_3$	(*E*)-Octadec-8-enyl	H	3.3 ± 0.4	1.4 ± 0.2	18.0 ± 3.5
	21	H	n-$C_{14}H_{29}$	H	1.9 ± 0.6	0.6 ± 0.1	2.8 ± 0.2
	22	H	n-$C_{16}H_{33}$	H	1.9 ± 0.1	0.7 ± 0.1	2.2 ± 0.2
	23	H	OCH_3	CH_3	>100	>100	>100
	26	NH_2	n-$C_{12}H_{25}$	H	2.2 ± 0.1	1.4 ± 0.1	4.1 ± 0.5
	27	NH_2	n-$C_{16}H_{33}$	H	2.3 ± 0.1	1.4 ± 0.1	7.4 ± 1.1
	**24**	3,4,5-Trimethoxyl	n-$C_{14}H_{29}$	H	2.6 ± 0.2	1.1 ± 0.1	4.1 ± 0.7
	25	$NHCOCH_3$	n-$C_{16}H_{33}$	H	3.2 ± 0.2	1.4 ± 0.1	22.6 ± 3.0
	**28**	H	n-$C_{14}H_{29}$	H	42.1 ± 5.0	>50	>50
	29	3,4-Dimethoxyl	n-$C_{14}H_{29}$	H	19.1 ± 1.8	42.9 ± 10.1	>50
	**30**	$NHCOCH_3$	n-$C_{16}H_{33}$	H	29.1 ± 1.0	>100	>100
	**31**	3,4,5-Trimethoxyl	N/A	N/A	>50	>50	>50
	DTIC				>100	>100	ND
	Sorafenib				5.4 ± 0.5	4.9 ± 0.3	15.1 ± 1.2[a]

IC_{50} values expressed with standard error.

[a] This represents four repeated measurements on a new batch of fibroblast cells spanning 5th to 7th passages for sorafenib. Our previous result (>100 μM on fibroblast cells, see Ref. 24) was obtained on an old batch of fibroblast cells spanning 10th to 12th passages. Unlike cancer cells, older batch of fibroblast cells were no longer viable beyond their 14th passage. We also repeated bioassay for those active compounds (e.g., **17**, **20Z**, **20E** in the table) along with sorafenib using the new fibroblast cells. The results from our compounds are very consistent with those obtained from the old batch of fibroblast cells, indicating that old fibroblast cells somehow develop certain resistance to sorafenib.

tration which inhibited cell growth by 50% of DMSO-treated controls) values were obtained by nonlinear regression analysis with GraphPad Prism (GraphPad Software, San Diego, CA).

The ability of thiazolidine derivatives to inhibit the growth of two melanoma cancer cell lines and fibroblast cells is summarized in Table 1. Sorafenib (Velcade) has been used extensively in clinical trials for melanoma, hence we selected this compound and DTIC as reference standards to assess the activity of our compounds. At this early stage, all compounds were used as a diastereomeric mixture if they contain chiral centers in order to select the most promising compounds for further development.

Examination of antiproliferative effects for a variety of substitutions on the phenyl ring revealed the chain-length dependence for these compounds (**1**–**5**, **6**–**9**, **10**–**13**, **14**–**19**). Short chain length such as a C10 chain (for example, compounds **2**, **6**, **10**) displayed low potency for both cancer cells and fibroblast cells. As

chain length increased, potency increased, as well as toxicity as measured on fibroblast cells except when the acetyl amino group was substituted on the phenyl ring (compounds **14**–**17**). Both C15 and C16 chains with this substitution displayed both high potency and high selectivity against cancer cells, with an IC_{50} for melanoma cells as low as 600 nM (compound **17**). Further chain length increases, however, reduced potency and selectivity. At a chain length of C18 (compound **19**), the IC_{50} value was higher than 10 μM for all three cell lines. Interestingly, adding either a *cis*- or *trans*-double bond in the C18 side chain restored potency dramatically (compounds **20Z** and **20E**), demonstrating that both length and composition of the side chain are critical for their activity. There is no significant difference in their activity between the *cis*- and *trans*-isomers.

Removing the acetyl amino group on the phenyl ring (compounds **21** and **22**) resulted in the loss of selectivity, although potency was similar to those with this substitution (compounds **15** and **17**). Replacing the alkyl chain with a methoxyl group completely abolished potency (compound **23**). Changing the chirality from an *R* to *S* configuration at the C4 position on the thiazolidine ring did not substantially affect either potency or selectivity (compound **4** vs **24** and compound **17** vs **25**). Selectivity has a strong dependence on the substitutions in the phenyl ring. For example, with a C12 chain, potency is similar for all the substitutions we studied (compounds **3**, **7**, **11**, **14**, and **26**). However, selectivity improves dramatically when proper substitutions are present (compound **17** vs compounds **5**, **9**, **13**, **22**, and **27**).

When the amino group in the thiazolidine ring is substituted (compound **30**) or a double bond is introduced (compounds **28** and **29**), the resulting compounds are largely inactive with IC_{50} values above 20 μM. We also tested the intermediate compound in which the amino group is protected by a Boc group, and that compound is inactive also (data not shown). Furthermore, when we removed the aliphatic chain and the amino group by synthesizing a dimer, we obtained an inactive compound (compound **31**). These results clearly demonstrate the importance of the amino group in the thiazolidine ring.

Not surprising, DTIC was inactive ($IC_{50} > 100$ μM) in our in vitro assay due to lack of bioactivation.[27] Recent clinical trials indicated that sorafenib has promising effect against melanoma, and it has very low toxicity.[28] Our in vitro assay indicated that compound **17** was more potent and selective against melanoma cells than sorafenib, as indicated by the ratio of its IC_{50} values for fibroblast cells over melanoma cells (10–20 for **17** vs 3–4 for sorafenib).

In conclusion, we have synthesized novel analogs of thiazolidine compounds based on initial studies. When compared with existing anticancer drugs, our compounds were more potent and selective. Further optimization of the structure to improve their activities is currently in progress. Once highly potent and selective compounds are identified, pure optical isomers will be separated by preparative HPLC for both in vitro and in vivo animal testing.

Acknowledgments

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