Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5289–5293
Bioorganic & Medicinal Chemistry Letters

Synthesis and antiproliferative activity of 2-aryl-4-oxo-thiazolidin-3-yl-amides for prostate cancer☆

Veeresa Gududuru,[a] Eunju Hurh,[b] 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*

Received 22 July 2004; revised 11 August 2004; accepted 11 August 2004
Available online 17 September 2004

Abstract—We have previously described serine amide phosphates (SAPs) as a novel class of cytotoxic agents for prostate cancer. Several of them showed potent cytotoxicity against human prostate cancer cell lines, but were not selective in non-tumor cells. To improve the selectivity and further enhance the potency, we designed a new series of 2-aryl-4-oxo-thiazolidin-3-yl amides. The current work describes synthesis, SAR, and biological evaluation of these compounds for their ability to inhibit the growth of prostate cancer cells. The antiproliferative effects of synthesized compounds were examined in five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, and TSU), and in RH7777 cells (negative controls). From this study, three potent compounds (**8**, **20**, and **21**) have been detected, which are effective in killing prostate cancer cells with improved selectivity compared to SAPs.

Prostate cancer accounts for 33% of all newly diagnosed malignancies among men in the United States.[1] According to the American Cancer Society,[2] an estimated 230,110 men will be diagnosed with prostate cancer in 2004, and 29,900 men will die of it. The incidence of prostate cancer varies worldwide, with the highest rates found in the United States, Canada, and Scandinavia, and the lowest rates found in China and other parts of Asia.[3,4] These differences are caused by genetic susceptibility, exposure to unknown external risk factor, or differences in health care and cancer registration, or even a combination of these factors.[4]

Cancer of the prostate is multifocal and it is commonly observed that the cancerous gland contains multiple independent lesions, suggesting the heterogeneity of the disease.[5] Determinants responsible for the pathologic growth of the prostate remain poorly understood, although steroidal androgens and peptide growth factors have been implicated.[6,7] As long as the cancer is confined to the prostate, it can be successfully controlled by surgery or radiation, but in metastatic disease, few options are available beyond androgen ablation,[8] the mainstay of treatment in the case of lymph node involvement or disseminated loci. Once tumor cells have become hormone refractory, the standard cytotoxic agents are marginally effective in slowing disease progression, although they do provide some degree of palliative relief. Current chemotherapeutic regimens, typically two or more agents, afford response rates in the range of only 20–30%.[9,10]

We have endeavored to address the need for improved antitumor therapy by means of a novel approach, and a recent report from our laboratory details the development of serine amide phosphates (SAPs), derivatives of lysophosphatidic acid (LPA) as effective cytotoxic agents against human prostate cancer cell lines.[11] We showed that SAP derivatives represent a class of anticancer agents for the treatment of prostate cancer and several of these were potent inhibitors of prostate tumor cell proliferation at low micromolar concentrations.[11] Despite their high cytotoxicity, the same compounds were not selective against non-tumor CHO cells and LPA receptor negative RH7777 cells. We also hypothesize that the phosphate group in SAPs is susceptible to hydrolysis, as the phosphate moiety is readily hydrolyzed by the action of lipid phosphate

Keywords: Prostate cancer; Lysophosphatidic acid; Phosphate mimics; Thiazolidinones; Antiproliferative effect.
☆This work was presented at the 31st MALTO Meeting, Memphis, TN, May 16–18, 2004.
*Corresponding author. Tel.: +1 901 448 6026; fax: +1 901 448 3446; e-mail: dmiller@utmem.edu

0960-894X/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2004.08.029

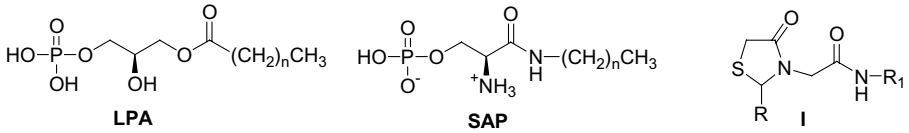
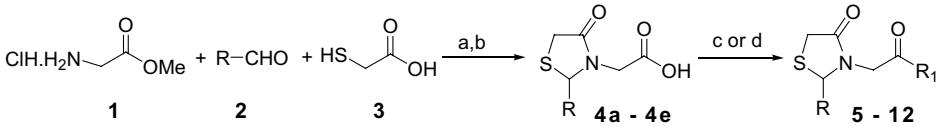


Figure 1.

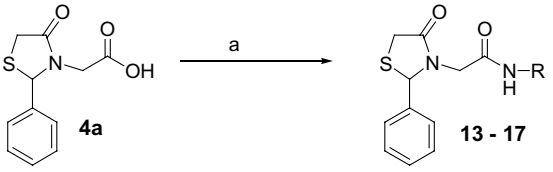


Scheme 1. Reagents: (a) toluene, Dean–Stark; (b) NaOH, MeOH; (c) $CH_3(CH_2)_nNH_2$, EDC, HOBt, CH_2Cl_2; (d) $(COCl)_2$, benzene, NH_3, MeOH.

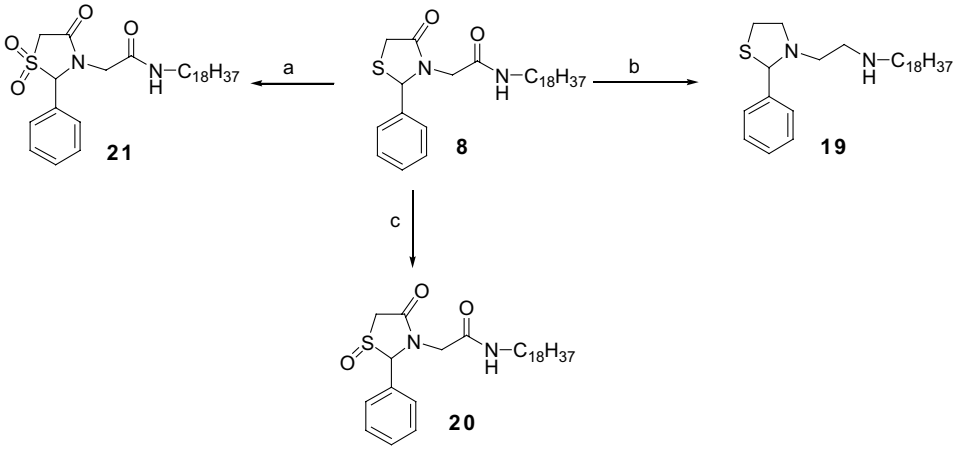
phosphatases.[12–14] The biomimetic replacement of phosphate group by a hydrolytically more stable pharmacophore would be expected to prolong biological activity by altering pharmacokinetics and metabolism.

Andres et al. have postulated that 4-thiazolidinones may be recognized as phosphate mimics, and using this scaffold they synthesized and evaluated some 4-thiazolidinones for their ability to inhibit the bacterial enzyme MurB.[15] Based on this hypothesis, we decided to explore the 4-thiazolidinone pharmacophore as a biomimetic replacement for the phosphate group. This strategic modification would be expected to enhance the physiochemical, pharmacokinetic, and antiproliferative properties and result in highly potent and selective anticancer agents for prostate cancer. To this end, we designed a new series of thiazolidinone derivatives as shown in Figure 1. In this paper we report the synthesis, structure–activity relationship, and antiproliferative activity of type **I** compounds (Fig. 1) in five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, and TSU).

The synthesis of thiazolidinone derivatives (**5**–**12**) utilized straightforward chemistry as shown in Scheme 1. Various 4-thiazolidinones were synthesized following a reported procedure[16] of condensing mercaptoacetic acid, glycine methyl ester, and aromatic aldehydes in a one-pot reaction, followed by basic hydrolysis of the ester. Thiazolidinone amides were obtained by the treatment with appropriate amines in the presence of EDC/HOBt under standard conditions. Compound **5** that has no side chain was synthesized from the corresponding acid as shown in Scheme 1. Thiazolidinone amides **13**–**17** were synthesized by a simple and direct method,[17] which involves reaction of the acid **4a** with different isocyanates in the presence of a catalytic amount of DMAP (Scheme 2). Exhaustive reduction of **8** using BH_3·THF under reflux conditions gave **19** (Scheme 3). Oxidation of **8** using H_2O_2 and with $KMnO_4$ afforded sulfoxide (**20**) and sulfone (**21**), respectively, as shown in Scheme 3. All compounds[18] were characterized by ^1H and ^{13}C NMR, mass spectroscopy and, in certain cases, elemental analysis.

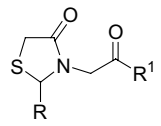


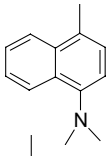
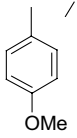
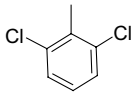
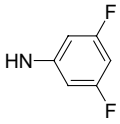
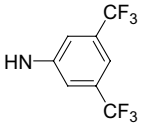
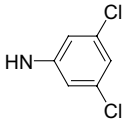
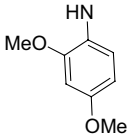
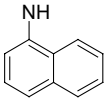
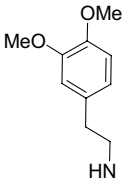
Scheme 2. Reagents: (a) R–NCO, DMAP, CH_2Cl_2.



Scheme 3. Reagents: (a) $KMnO_4$, AcOH; (b) BH_3·THF; (c) H_2O_2, AcOH.

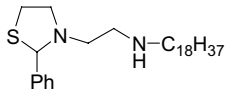
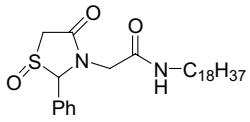
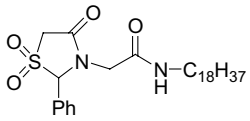
Table 1. Antiproliferative effects of compounds **4a**–**b** and **5**–**18**



Compd			IC_{50} (μM)					
	R	R^1	RH 7777[a]	DU-145[b]	PC-3[b]	LNCaP[b]	PPC-1[b]	TSU[b]
4a	Ph	OH	ND	>50	>50	>50	>50	>50
4b	Biphenyl	OH	>100	>100	>100	>100	>100	>100
5	Ph	NH_2	>100	>100	>100	>100	>100	>100
6	Ph	$NHC_{10}H_{21}$	20.0	22.4	20.3	14.1	15.8	19.7
7	Ph	$NHC_{14}H_{29}$	16.4	19.6	13.5	14.1	10.1	13.4
8	Ph	$NHC_{18}H_{37}$	39.6	12.6	11.1	9.3	7.1	8.5
9	Biphenyl	$NHC_{18}H_{37}$	>50	>50	>50	>50	>50	>50
10		$NHC_{18}H_{37}$	>50	>50	>50	>50	>50	>50
11		$NHC_{18}H_{37}$	31.1	14.8	12.6	11.8	10.7	17.5
12		$NHC_{18}H_{37}$	>50	>50	>50	>50	>50	>50
13	Ph		70.9	69.0	74.1	24.1	46.2	53.2
14	Ph		25.4	16.2	18.1	14.5	13.1	16.1
15	Ph		34.9	24.0	28.6	13.2	20.5	17.2
16	Ph		>100	>100	>100	82.5	>100	60.8
17	Ph		>100	>100	>100	31.4	>100	69.9
18	Ph		>100	>100	>100	>100	>100	>100
	5-FU		ND	11.9	12.0	4.9	6.4	3.6

[a] Control cell line.
[b] Prostate cancer cell lines.

Table 2. Antiproliferative effects of compounds **19–21**

Compd	Structure	IC_{50} (μM)					
		RH 7777[a]	DU-145[b]	PC-3[b]	LNCaP[b]	PPC-1[b]	TSU[b]
19		>20	15.8	>20	>20	12.0	6.1
20		11.5	11.2	6.5	7.9	5.4	6.4
21		22.1	15.5	8.5	10.9	5.5	9.3
	5-FU	ND	11.9	12.0	4.9	6.4	3.6

[a] Control cell line.
[b] Prostate cancer cell lines.

The antiproliferative activity of all the synthesized compounds has been evaluated against five human prostate cancer cell lines and in RH7777 cells (negative control) using the sulforhodamine B (SRB) assay.[19] 5-Fluorouracil (5-FU) was used as reference drug. As shown in Table 1, 4-thiazolidinone carboxylic acids (**4a** and **4b**) were unable to inhibit the growth of any of the five prostate cancer cells below 50 μM. However, the corresponding amides (**6–8**) showed higher activities. It was observed that an increase in the alkyl chain length [**6** (C10), **7** (C14), and **8** (C18)] enhances the antiproliferative activity of these analogs in prostate cancer cells. Interestingly, the simple amide **5** without any long alkyl chain is not cytotoxic below 100 μM, which indicates that the absence of an alkyl side chain causes a considerable decrease in antiproliferative effect. On the other hand, replacement of the alkyl chain with various aryl side chains (**13–18**) reduced the biological activity. Among this series, **13** is moderately cytotoxic, where as analogs **16–18** displayed poor cytotoxicity in several prostate cancer cell lines. However, it is noteworthy to mention that thiazolidinone amides (**14** and **15**), with electron-withdrawing substituents on the aryl ring showed cytotoxicity in the range of 13–29 μM against all five prostate cancer cell lines.

Thiazolidinone derivatives (**9** and **10**) with bulky biphenyl or naphthalene groups demonstrated low cytotoxicity compared to **8** (Table 1). We synthesized compounds **11** and **12** to understand the effects of aromatic ring substitution in **8**. It was observed that electron-donating substituents maintained good activity while the *ortho* electron-withdrawing substituents substantially decrease the antiproliferative activity of these derivatives (Table 1). Compound **19**, which has no amide groups, showed significantly good potency in all five prostate cancer cell lines. Notably, **20** and **21** bearing sulfoxide or sulfone moiety displayed higher cytotoxic potency comparable to that of the reference drug 5-FU against both PC-3 and PPC-1 cell lines (Table 2).

In summary, starting with the SAPs, we identified a series of novel and cytotoxic 4-thiazolidinone amides based on a 4-thiazolidinone scaffold. Among this series, we synthesized and carried out detailed structure activity relationship studies of type **I** compounds (Fig. 1) and evaluated their antipropliferative activity against five prostate cancer cell lines and RH7777 cells (negative controls). The cytotoxicity study shows that the antiproliferative activity is sensitive to 2-aryl ring substitutions, the length of the alkyl side chain, and the removal or replacements of the lipophilic alkyl side chain. Sulfur oxidation is well tolerated as compounds **20** and **21** showed significant cytotoxicity compared to 5-FU. This study resulted in the discovery of potent cytotoxic 4-thiazolidinones **8**, **20**, and **21**, which inhibit the growth of all five human prostate cancer cell lines (DU-145, PC-3, LNCaP, PPC-1, and TSU) with 2–5-fold lower selectivity compared to RH7777 cell line. These 4-thiazolidinone derivatives are a significant improvement on the SAP moiety in that they are less cytotoxic but demonstrated improved selectivity in non-tumor cells. However, further investigations are required to increase the potency and selectivity.

Acknowledgements

This research was supported by a grant from the Department of Defense (DAMD17-01-1-083).

References and notes

1. American Cancer Society: *Cancer Facts and Figures,* 2003; American Cancer Society: Atlanta, GA, 2003.
2. American Cancer Society: *Cancer Facts and Figures*, 2004.
3. Quinn, M.; Babb, P. *BJU Int.* **2002**, *90*, 162.
4. Gronberg, H. *Lancet* **2003**, *361*, 859.

5. Foster, C. S.; Bostwick, D. G.; Bonkhoff, H.; Damber, J. E.; van der Kwast, T.; Montironi, R.; Sakr, W. A. *Scand. J. Urol. Nephrol.* **2000**, *205*, 19.
6. Agus, D. B.; Cordon-Cardo, C.; Fox, W.; Drobnjak, M.; Koff, A.; Golde, D. W.; Scher, H. I. *J. Natl. Cancer. Inst.* **1999**, *91*, 1869.
7. Djakiew, D. *Prostate* **2000**, *42*, 150.
8. Frydenberg, M.; Stricker, P. D.; Kaye, K. W. *Lancet* **1997**, *349*, 1681.
9. Beedassy, A.; Cardi, G. *Sem. Oncol.* **1999**, *26*, 428.
10. Raghavan, D.; Koczwara, B.; Javis, M. *Eur. J. Cancer* **1997**, *33*, 566.
11. Gududuru, V.; Hurh, E.; Durgam, G. G.; Hong, S. S.; Sardar, V. M.; Xu, H.; Dalton, J. T.; Miller, D. D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4919.
12. Jasinska, R.; Zhang, Q. X.; Pilquil, C.; Singh, I.; Xu, J.; Dewald, J.; Dillon, D. A.; Berthiaume, L. G.; Carman, G. M.; Waggoner, D. W.; Brindley, D. N. *Biochem. J.* **1999**, *340*, 677.
13. Xu, J.; Love, L. M.; Singh, I.; Zhang, Q. X.; Dewald, J.; Wang, D. A.; Fischer, D. J.; Tigyi, G.; Berthiaume, L. G.; Waggoner, D. W.; Brindley, D. N. *J. Biol. Chem.* **2000**, *275*, 27520.
14. Hooks, S. B.; Santos, W. L.; Im, D. S.; Heise, C. E.; Macdonald, T. L.; Lynch, K. R. *J. Biol. Chem.* **2001**, *276*, 4611.
15. Andres, C. J.; Bronson, J. J.; D'Andrea, S. V.; Deshpande, M. S.; Falk, P. J.; Young, K. A.; Harte, W. E.; Ho, H. T.; Misco, P. F.; Robertson, J. G.; Stock, D.; Sun, Y.; Walsh, A. W. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 715.
16. Homes, C. P.; Chinn, J. P.; Look, C. G.; Gordon, E. M.; Gallop, M. A. *J. Org. Chem.* **1995**, *60*, 7328.
17. Schuemacher, A. C.; Hoffman, R. W. *Synthesis* **2001**, *22*, 243.
18. Compounds were obtained as mixtures of diastereomers and were used as such for the biological studies. Characteristic data for some compounds are given below. Compound **8**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.0 Hz, 3H), 1.26 (br s, 30H), 1.46 (m, 2H), 3.16–3.29 (m, 3H), 3.82 (d, J = 1.5 Hz, 2H), 4.20 (s, 0.5H), 4.25 (s, 0.5H), 5.83–5.85 (m, 2H), 7.27–7.41 (m, 5H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 13.55, 22.13, 26.30, 28.69, 28.80, 28.88, 28.99, 29.03, 29.10, 29.14, 31.37, 32.13, 39.08, 45.88, 63.67, 127.05, 128.58, 128.96, 137.61, 166.30, 171.61; MS (ESI) m/z 511 [M+Na]. Anal. Calcd for $C_{29}H_{48}N_2O_2S$: C, 71.26; H, 9.90; N, 5.73. Found: C, 71.18; H, 10.03; N, 5.79. Compound **11**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.0 Hz, 3H), 1.26 (br s, 30H), 1.33 (s, 2H), 3.16–3.19 (m, 1H), 3.2–3.29 (m, 2H), 3.80 (d, J = 0.9 Hz, 2H), 3.83 (s, 3H), 4.16 (s, 0.5H), 4.21 (s, 0.47H), 5.82 (s, 1H), 6.9 (dd, J = 1.8 Hz, 2H), 7.29 (dd, J = 1.5 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 13.53, 22.12, 26.31, 28.70, 28.74, 28.79, 28.89, 28.99, 29.03, 29.09, 29.13, 31.36, 32.23, 39.06, 45.74, 54.79, 63.44, 128.64, 129.11, 159.97, 166.41, 171.47; MS (ESI) m/z 541 [M+Na]. Anal. Calcd for $C_{30}H_{50}N_2O_3S$: C, 69.45; H, 9.71; N, 5.40. Found: C, 69.30; H, 9.86; N, 5.43. Compound **12**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.54 (d, J = 15.3 Hz, 1H), 3.87 (s, 2H), 4.25 (d, J = 15.3 Hz, 1H), 5.88 (s, 1H), 7.10 (t, J = 1.8 Hz, 1H), 7.36–7.43 (m, 7H), 8.29 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 32.35, 46.73, 64.40, 117.37, 123.85, 127.29, 128.74, 129.32, 134.59, 136.87, 138.61, 165.14, 172.60; MS (ESI) m/z 403 [M+Na]. Anal. Calcd for $C_{17}H_{14}Cl_2N_2O_2S$: C, 53.55; H, 3.70; N, 7.35. Found: C, 53.39; H, 3.47; N, 7.36. Compound **21**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.0 Hz, 3H), 1.26 (br s, 32H), 3.19–3.34 (m, 3H), 3.88–4.03 (dd, J = 16.5 Hz, 2H), 4.66 (s, 0.5H), 4.72 (s, 0.5H), 5.67 (br s, 1H), 5.95 (s, 1H), 7.38 (m, 2H), 7.50–7.53 (m, 3H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 13.54, 22.12, 26.26, 28.66, 28.79, 28.96, 29.02, 29.09, 29.14, 31.36, 39.30, 44.35, 49.85, 81.32, 125.77, 128.43, 128.91, 130.55, 163.23, 165.30; MS (ESI) m/z 519 [M−H]. Anal. Calcd for $C_{29}H_{48}N_2O_4S$: C, 66.88; H, 9.29; N, 5.38. Found: C, 66.68; H, 9.27; N, 5.41.
19. Thiazolidinone derivatives were dissolved in dimethyl sulfoxide (DMSO) and serially diluted in complete growth medium to desired final concentrations at DMSO concentrations of less than 0.5%. Cells were exposed to a wide range of concentrations (0–100 μM) of the particular compound for 96 h in 96 well plates. Cells were fixed with 10% trichloroacetic acid and washed five times with water. The plates were air dried overnight and fixed cells were stained with SRB solution. The cellular protein-bound SRB was measured at 540 nm using a plate reader. Cell numbers at the end of the treatment were measured. IC_{50} (i.e., concentration that inhibited cell growth by 50% of untreated control) values were obtained by nonlinear regression analysis using WinNonlin.