Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5072–5077

Identification of Sansalvamide a analog potent against pancreatic cancer cell lines

Po-Shen Pan,[a] Kathleen L. McGuire[b] and Shelli R. McAlpine[a,*]

[a]*Department of Chemistry and Biochemistry, San Diego State University, San Diego, CA 92182, USA*
[b]*Department of Biology, San Diego State University, San Diego, CA 92182, USA*

Received 1 May 2007; revised 3 July 2007; accepted 6 July 2007
Available online 19 July 2007

Abstract—Thirty-one Sansalvamide A peptide derivatives were synthesized. ^{3}H thymidine inhibition assays were performed using two pancreatic cancer cell lines (PL45 and BxPC-3). Six compounds possess 140-fold increased differential selectivity for cancer cell lines over normal cell lines (WS1, skin fiberblasts) and are 140 times more active against pancreatic cancer cell lines than compounds used clinically to treat these cancers (e.g., 5-FU). Structure–activity relationship studies show the inclusion of a single *N*-methyl and/or D-amino acid appears to be critical for presenting the active conformation of the six San A peptide derivatives to their biological target(s).

Pancreatic cancer is the fifth most deadly cancer in the U.S. Only 10% of patients are eligible for surgery,[1] and less than 20% of pancreatic cancers respond to the drug of choice [2,2-difluorodeoxycytidine] or other drugs on the market.[2] The 5-year survival rate for patients with pancreatic cancers is less than 5% (95% mortality rate!).[3] With such a low response rate to current chemotherapeutic treatments, there is an immediate need for new drugs that provide additional chemotherapeutic options to patients.

Pioneering work by Silverman on the synthesis of Sansalvamide A peptide (San A) brought attention to this class of natural product analogs.[4,5] Silverman describes the cytotoxicity of eleven Sansalvamide A (San A) derivatives against drug-resistant cell line HCT-116.[3,4] We recently reported the synthesis and cytotoxicity data of 70 derivatives against HT-29,[6,7] a drug-sensitive colon cancer cell line, and HCT-116.[8] The natural product is a depsipeptide, and as such is prone to deactivation by ring opening enzymes. In order to avoid this, most of the 81 analogs synthesized, including all 70 of those reported by our laboratory, were synthesized as the San A peptide derivatives. Although one San A derivative has been reported by Silverman as potent against pancreatic cancers,[9] this is the first report to describe the SAR of our derivatives against pancreatic cancer cell lines. Therefore in this communication we highlight the key SARs for targeting these drug-resistant pancreatic cancers. We describe the first extensive SAR study of 31 San A derivatives in *two* distinguishable pancreatic cancer cell lines.[10] Six structurally unique compounds emerge as potent inhibitors.

^{3}H thymidine inhibition assays using PL45 and BxPC-3 pancreatic cancer cell lines highlight the extraordinary promise of these six San A derivatives and provide valuable data essential in designing new chemotherapeutic agents against pancreatic cancers. Our small molecules share no homology to known pancreatic cancer drugs, demonstrate selectivity for cancer cell lines over non-cancerous cell lines, have C log *P* values within the range of Lipinski's rules (0.18–3.3), and show potency on par with current drugs on the market treating other cancers. These data establish that San A is a privileged structure for treating multiple drug-resistant pancreatic cancers. Our succinct convergent approach provided 31 analogs.[6,7] We describe how our derivatives provide valuable information on the impact of: stereochemistry, amide bond geometry, hydrophobic moieties, and hydrophilic elements on potency. In addition, choices of amino acids were designed to alter the C log *P* values in the range of 0.13–3.5, thus exploring solubility properties while still meeting Lipinski's rules. Compounds

Keywords: Pancreatic cancer; Macrocyclic peptide; Macrocycle; D-amino acid; Conformation; Pentapeptide; Antitumor; Sansalvamide A; Peptide; Pancreatic cancer.
* Corresponding author. Tel.: +1 619 594 5580; fax: +1 619 594 4634; e-mail: mcalpine@sciences.sdsu.edu

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.07.025

are described by modifications at each position. Data shown below give the percent inhibition at 5 μM concentrations. We synthesized the all-peptide natural product analog [compound (**1**)] for comparison to our macrocyclic peptides. Figure 1 shows that two alterations at position 1 have an impact on cytotoxicity. Compound (**2**), which contains a D-amino acid (D-aa), and compound (**3**), which contains an *N*-methyl, moiety are both relatively potent. These two compounds with modifications at 1 demonstrate an improvement in growth inhibition over that of the natural product peptide (**1**).

Changes at position 2 have no effect on cytotoxicity with all compounds being as ineffective as (**1**) (Fig. 2). Interestingly, placement of an *N*-methyl [(**6**)], a D-leucine [(**7**)], *N*-methyl D-leucine [(**8**)], a hydrophobic D-phenylalanine [(**9**)], or the polar residue serine [(**10**)] at position 2 had little impact on potency.

Of all five positions, position 3 seems to have the greatest impact on cytotoxicity (Fig. 3). Placement of a single D-valine at position 3 [compound (**12**)] produces an extraordinarily potent compound against both pancreatic cancer cell lines. Yet, an *N*-methyl or *N*-methyl-D-valine at this position [compounds (**11**) and (**14**), respectively] decreases cytotoxicity relative to San. A (**1**). Interestingly, placement of an *N*-methyl D-valine at 3, combined with a hydrophobic cyclohexyl (**15**) provides a relatively potent compound. In contrast, an *N*-methyl D-valine at 3 combined with a D-leucine at 5 generates a non-potent compound [(**20**)]. Yet, substitution of a relatively hydrophobic element such as D-phenylalanine [(**17**)], or a polar element such as D-serine [(**16**)] at position 3 both diminish the compounds' activity relative to San A. In addition, the substitution of a D-ethyl moiety at 3 (**18**), unlike compound (**12**), produces an ineffective compound. The combination of a D-valine at position 3 and either a D-leucine at 2 [(**19**)], or a D-leucine at position 5 [(**21**)], or an *N*-methyl at 5 [(**22**)] generates compounds that are less potent than compounds containing only D-valine at position 3 [(**12**)]. Finally, two analogs containing a D-valine at 3 and a lysine-protected moiety at 4 [(**24**)], or an unprotected lysine at 4 [(**23**)] both exhibit minimal cytotoxicity. In summary, compounds (**12**) and (**15**) show the greatest potency against pancreatic cancer cell lines. Overall one compound with modifications at position 3, compound (**12**), demonstrated a clear and significant improvement in growth inhibition maintaining greater than 50% against both cell lines.

Changes at position 4 appear to have some impact on activity, where the inclusion of an *N*-methyl moiety [(**26**)] improves cytotoxicity relative to San A (Fig. 4) for PL45 but not BxPC-3. Yet, an *N*-methyl moiety at 4 combined with a D-aa at 5 shows cytotoxicity against BxPC-3 and not PL45 (**27**). A D-aa at 4 improves cytotoxicity for BxPC-3 and not PL45 (**25**). Finally, a D-amino acid in positions 4 and 5 (**28**) greatly enhances potency for both compounds relative to San A. As mentioned earlier, (**15**), which contains a D-aa at 3 and a cyclohexyl at 4, is relatively potent against both cell lines. Thus, only one compound had growth inhibition percentages greater than 50% in both cell lines: (**28**). This compound showed a significant increase in cytotoxicity over (**1**).

Position 5 also appears to have an impact on cytotoxicity where placement of a single *N*-methyl at 5 [(**29**)]

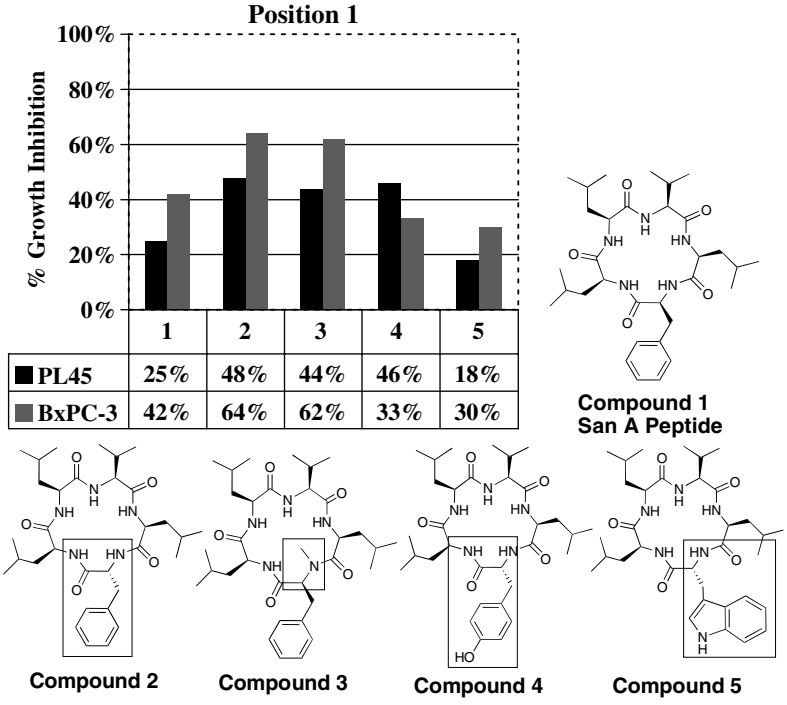

Figure 1. Compounds with changes at position 1. Each data point is an average of three wells from three assays at 5 μM. Error = ±5%, DMSO was used as a control.

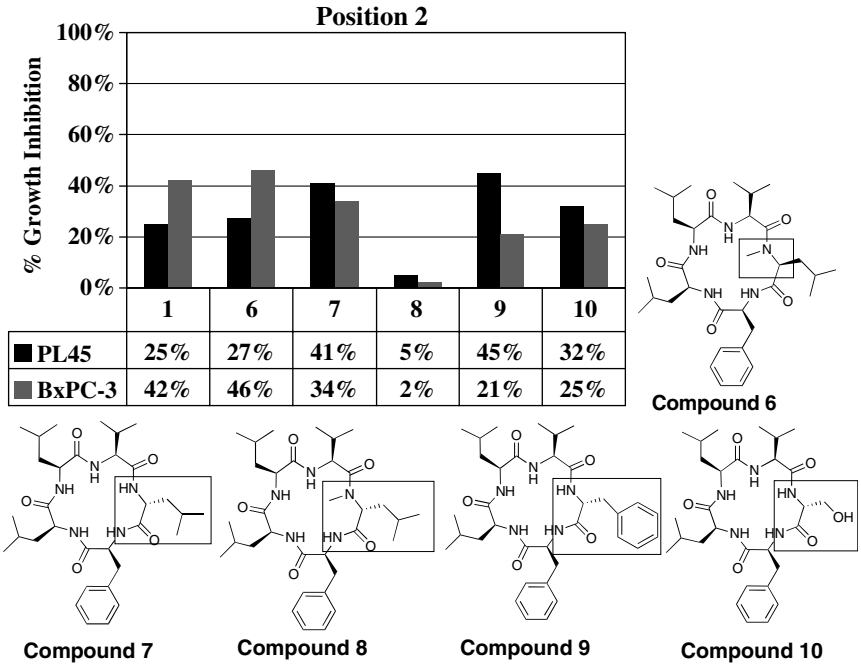

Figure 2. Compounds with changes at position 2. Each data point is an average of three wells run in three assays at 5 μM. Margin of error = ±5%, DMSO was used as a control.

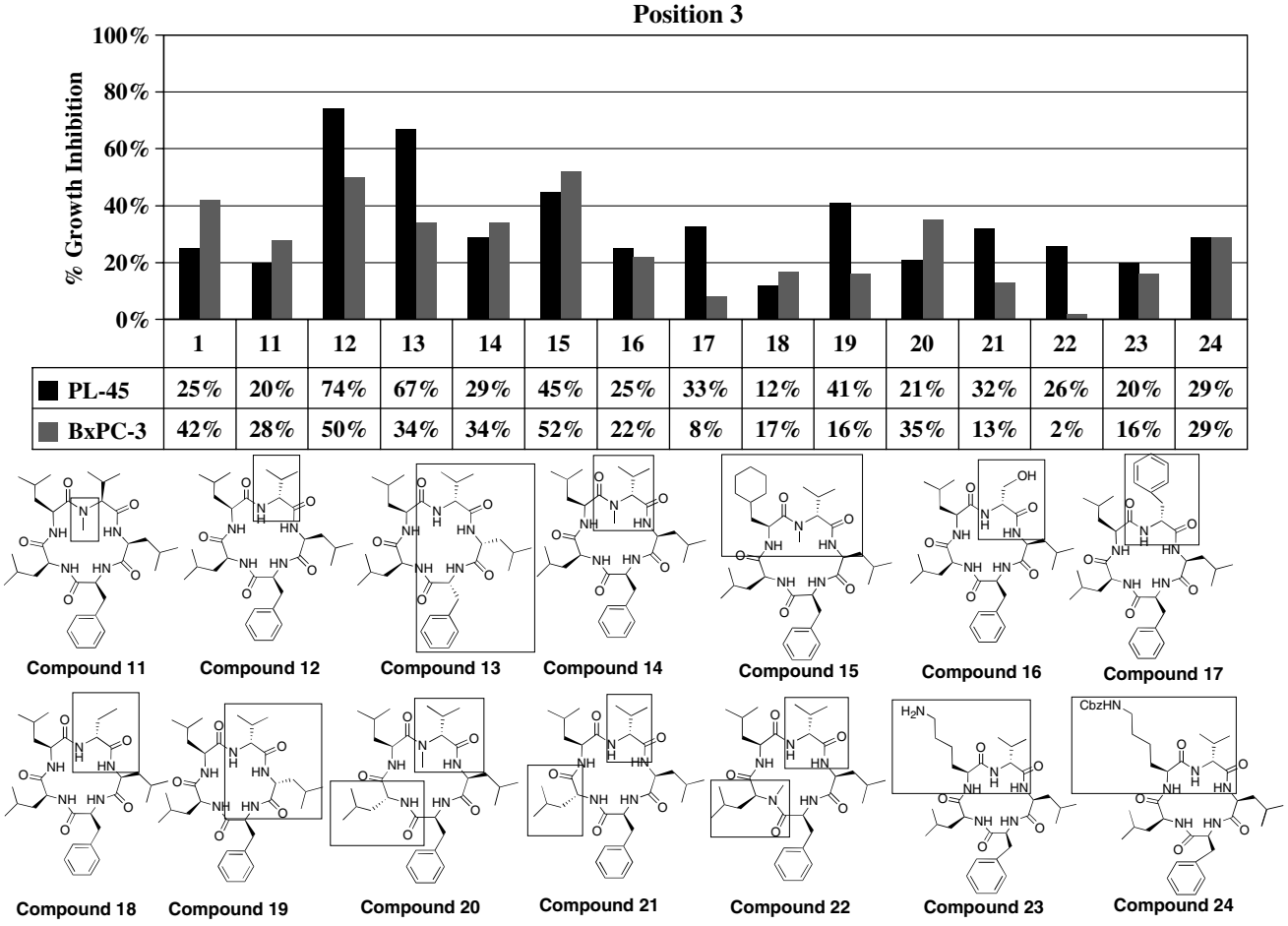

Figure 3. Compounds with changes at position 3. Each data point is an average of three wells run in three assays at 5 μM. Margin of error = ±5%, DMSO was used as control.

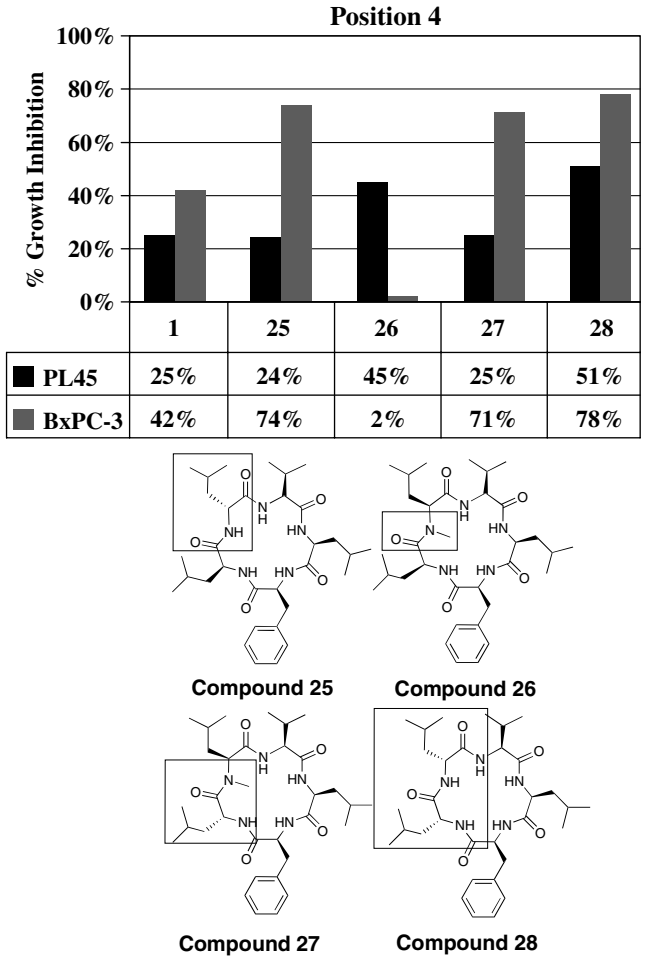

Figure 4. Compounds with changes at position 4. Each data point is an average of three wells run in three assays. Margin of error = ±5%, DMSO was used as control.

manifests greater cytotoxicity than San A (Fig. 5). A D-leucine at 5 shows potency against PL45 but not BxPC-3 [(**30**)]. Interestingly a combination of an *N*-methyl D-aa at 5 [(**31**)] shows similar potency to that of compound (**29**), which only has an *N*-methyl at 5. In addition, compound (**21**), which is a combination of potent compounds (**12**) and (**30**), is not potent against either cell line. Similarly a combination of potent compounds (**12**) and (**29**) generates an ineffective compound [(**22**)]. Overall, two compounds, (**29**) and (**31**), showed significant potency against both cell lines. They displayed growth inhibition percentages greater than 50% in both cell lines.

The IC_{50} values for the six most potent compounds are shown (Fig. 6). Importantly, all six compounds exhibit very low micromolar IC_{50} values for both cell lines. Two compounds, (**12**) and (**28**), are greater than 50-fold more potent than the natural product peptide (**1**) and possess ~140-fold increased differential selectivity for cancer cell lines versus normal cell lines (WS1, skin fiberblasts).

The most important features to emerge from this SAR study include the incorporation of a D-phenylalanine or *N*-methyl at position 1, a D-valine at 3, and an *N*-methyl or *N*-methyl D-leucine at 5. Thus, a single feature or position is not responsible for potency, rather as is typical in complex systems, there are several determining factors. The key connection between potency and structure involves constraining the macrocycle into its active conformation and thus binding to its biological target in that position. Recent publications highlighted that a *single* *N*-methyl D-amino acid (D-aa) was the central structural component required to maintain a dominant conformation in macrocycles with five amino

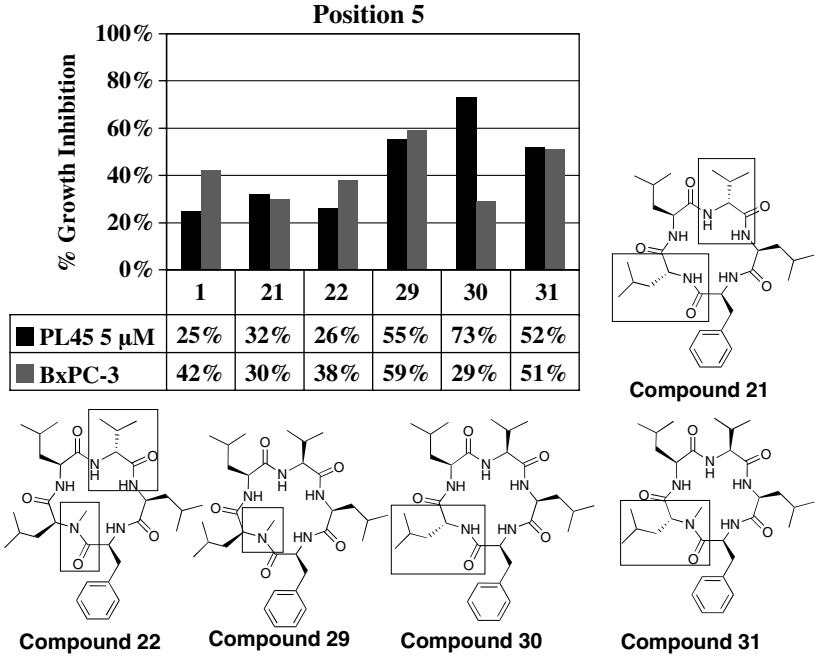

Figure 5. Compounds with changes at position 5. Each data point is an average of three wells run in three assays. Margin of error = ±5%, DMSO was used as control.

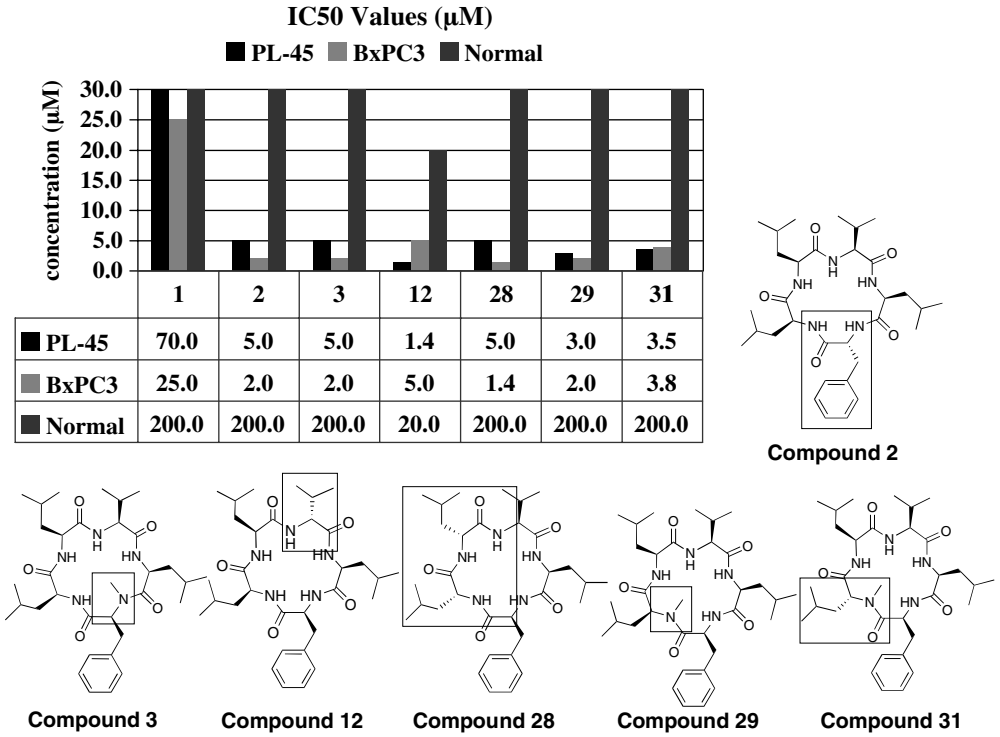

Figure 6. IC_{50}s of potent compounds. Each data point is an average of three wells run in three assays at four concentrations. Margin of error = ±5%, DMSO was used as control. Normal cells are WS1 skin fiberblasts.

acids.[11–13] Thus, the inclusion of a *single N*-methyl D-aa in our active structures locks the ring into its low energy conformation, allowing it to appropriately present its side-chains to its biological target(s). Further, it is well established that these cyclic pentapeptides serve as templates for appropriately positioning suitable binding motifs for proteins by mimicking beta and gamma turns.[14,15] Thus, the inclusion of a single *N*-methyl and/or D-aa, which is seen in five of the six most potent compounds, plays a significant role in presenting the active conformation of San A to its biological target. Molecular modeling studies utilizing NOE values for the potent compounds are currently underway and will be reported in the near future.

These data show that significant progress has been made toward development of efficacious compounds against pancreatic cancers. Specifically, we have identified six compounds that show 140-fold differential selectivity against pancreatic cancer cell lines and demonstrate 50-fold greater potency than the natural product peptide (**1**). These results establish the San A scaffold as a promising structure for the development of new antitumor agents and compounds incorporating important structural features are being synthesized. Their potency will be reported in due course.

Acknowledgments

We thank San Diego State University for financial support. Partial support was provided by SDSU-RSCA to K.L.M.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.025.

References and notes

1. Murr, M. M.; Sarr, M. G.; Oishi, A. J.; Heerden, J. A. *CA Cancer J. Clin.* **1994**, *44*, 304.
2. Burris, H. A.; Moore, M. J.; Andersen, J.; Greem, M. R.; Rothenberg, M. I.; Modiano, M. R.; Cripps, M. C.; Portenoy, R. K.; Sotorniolo, A. M.; Tarassaoff, P.; Nelson, R.; Dorr, F. A.; Stephens, C. D.; vonHoff, D. *J. Clin. Oncol.* **1997**, *15*, 2403.
3. Sener, S. F.; Fremgen, A.; Menck, H. R.; Winchester, D. P. *J. Am. Coll. Surg.* **1999**, *189*, 1.
4. Lee, Y.; Silverman, R. B. *Org. Lett.* **2000**, *2*, 3743.
5. Gu, W.; Liu, S.; Silverman, R. B. *Org. Lett.* **2002**, *4*, 4171.
6. Rodriguez, R.; Pan, P.-S.; Pan, C.-M.; Ravula, S.; Lapera, S.; Singh, E.; Styers, T. J.; Brown, J. D.; Cajica, J.; Parry, E.; Otrubova, K.; McAlpine, S. R. *J. Org. Chem.* **2007**, *72*, 1980.
7. Styers, T. J.; Kekec, A.; Rodriguez, R.; Brown, J. D.; Cajica, J.; Pan, P.-S.; Parry, E.; Carroll, C. L.; Medina, I.; Corral, R.; Lapera, S.; Otrubova, K.; Pan, C.-M.; McGuire, K. L.; McAlpine, S. R. *Bioorg. Med. Chem.* **2006**, *14*, 5625.
8. Otrubova, K.; Styers, T. J.; Pan, P.-S.; Rodriguez, R.; McGuire, K. L.; McAlpine, S. R. *Chem. Commun.* **2006**, 1033.
9. Ujiki, M.; Milam, B.; Ding, X.-Z.; Roginsky, A. B.; Salabat, M. R.; Talamonti, M. S.; Bell, R. H.; Gu, W.; Silverman, R. B.; Adrian, T. E. *Biochem. Biophys. Res. Commun.* **2006**, *340*, 1224.
10. Two cell lines were chosen to provide evidence that our compounds were targeting a type of cancer, that is,

pancreatic. PL45 is a primary pancreatic ductial adenocarcinoma and BxPC-3 is the cell line of choice for xenograft mouse model studies.
11. Chatterjee, J.; Mierke, D. F.; Kessler, H. *J. Am. Chem. Soc.* **2006**, *128*, 15164.
12. Heller, M.; Sukopp, M.; Tsomaia, N.; John, M.; Mierke, D. F.; Reif, B.; Kessler, H. *J. Am. Chem. Soc.* **2006**, *128*, 13806.
13. Zhang, X.; Nikiforovich, G. V.; Marshall, G. R. *J. Med. Chem.* **2007**, ASAP.
14. Tyndall, J. D.; Pfieiffer, B.; Abbenante, G.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 793.
15. Viles, J. H.; Mitchell, J. B. L. G. S.; Doyle, P. M.; Harris, C. J.; Sadler, P. J.; Thornton, J. M. *Eur. J. Biochem.* **1996**, *242*, 352.