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Letters

Scaffold Targeting Drug-Resistant Colon Cancers

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Abstract: We have identified five derivatives of the natural product sansalvamide A that are potent against multiple drug-resistant colon cancer cell lines. These analogs share no structural homology to current colon cancer drugs, are cytotoxic at levels on par with existing drugs treating other cancers, and demonstrate selectivity for drug-resistant colon cancer cell lines over noncancerous cell lines. Thus, we have established sansalvamide A as a privileged structure for treating multiple drug-resistant colon cancers.

The existing model of carcinogenesis in the colonrectum suggests that there are two different pathways leading to colon cancer. A total of 80−85% of colon cancers demonstrate *chromosomal* instability and are considered treatable using current chemotherapeutic drugs. The remaining 15−20% of colon cancers involve a loss in the DNA mismatch repair system and are drug-resistant colon cancers.[1,2] The two types of colon cancers are referred to as MSS[a] (microsatellite stable), for the drug-sensitive colon cancers, and MSI (microsatellite unstable), for the drug-resistant colon cancers. MSS (drug-sensitive) colon cancers respond to the drug of choice, 5-fluorouracil (5-FU; $IC_{50} = 5\ \mu M$), where as MSI (drug-resistant) colon cancers do not respond to known chemotherapeutic agents.[1,2] Thus, there is an immediate need for new drugs that provide alternatives for MSI colon cancer patients.

Pioneering work by Silverman on the synthesis of sansalvamide A peptide (San A, compound **1**) brought attention to this class of natural product analogs.[3] Silverman describes the cytotoxicity of 11 sansalvamide A (San A) derivatives against drug-resistant cell line HCT-116 (MSI).[3,4] We recently reported cytotoxicity data of 35 derivatives against HT-29 (MSS),[5,6] a drug-sensitive colon cancer cell line, and 14 derivatives against HCT116.[7,8] However, there is an incomplete understanding of the structure−activity relationship (SAR) in drug-resistant colon cancer cell lines, and it is crucial that we elucidate the key SARs for targeting drug-resistant (MSI) colon cancers. We describe the first extensive SAR study of 32 San A derivatives in *two* distinguishable drug-resistant (MSI) colon cancer cell lines. Five structurally unique compounds (**5**, **10**, **13**, **16**, and **32**) emerge as potent inhibitors.

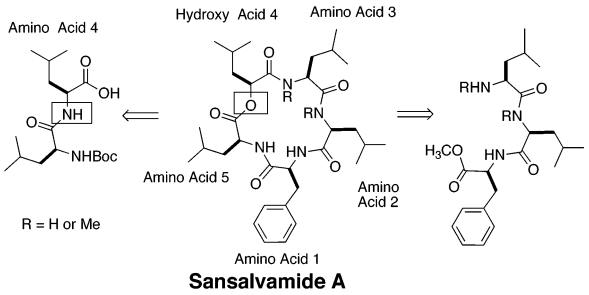

Figure 1. Retrosynthetic approach.

^{3}H thymidine inhibition assays highlight the extraordinary promise of these San A derivatives and provide valuable data essential in designing new chemotherapeutic agents against MSI colon cancers. Our small molecules share no homology to known colon cancer drugs, demonstrate selectivity for cancer cell lines over noncancerous cell lines, have ClogP 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 in treating multiple drug-resistant colon cancers.

Our succinct convergent approach (Figure 1) provided 32 analogues[5,6] This is the first time that cytotoxicity data is reported for these compounds in drug-resistant colon cancer cell lines.

Compounds are described by modifications at each position. ^{3}H-thymidine uptake assays were run using two distinguishable colon cancer cell lines: HCT-116 (MSI) and HCT-15 (MSI). Data shown below gives the percent inhibition at 1 μM concentrations. Potency exhibited by the San A peptide **1** is shown so that comparisons can be made between the natural product peptide and our synthetic analogs.

Figure 2 clearly shows that most alterations at position 1 do not produce a significant impact on cytotoxicity. The only

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[a] Abbreviations: MSS, microsatellite stable; MSI, microsatellite unstable.

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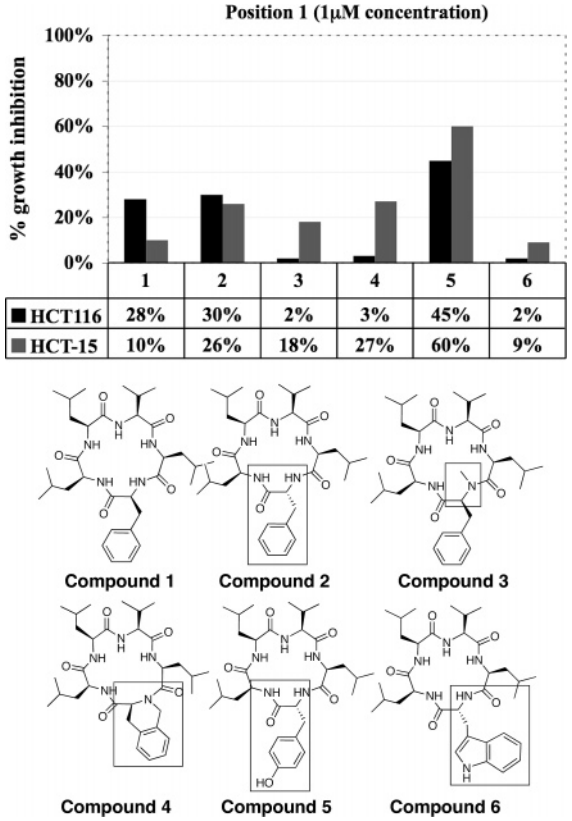

Figure 2. Compounds with changes at position 1. Each data point is an average of three wells run in two assays. Margin of error = ±5%.

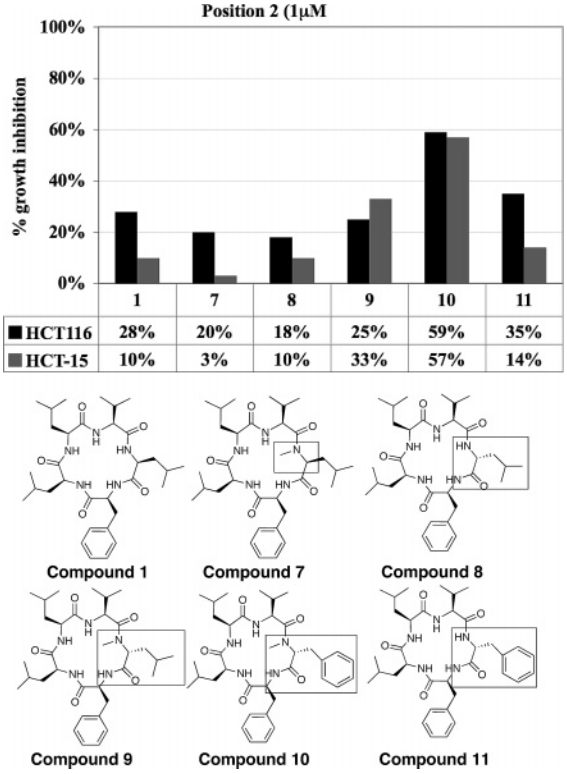

Figure 3. Compounds with changes at position 2. Each data point is an average of three wells run in two assays. Margin of error = ±5%.

exception is compound **5**, it appears to be relatively potent with an IC_{50} in the nanomolar range. Additional assays are underway with this lead compound, and compounds with tyrosine in position 1 are being incorporated into the third generation compounds.

Changes at position 2, however, clearly have an effect on cytotoxicity, with the *N*-methyl D-phenyl alanine derivative (compound **10**) producing exceptional potency (Figure 3). Interestingly, placement of an *N*-methyl (**7**), a D-leucine (**8**), an *N*-methyl L-leucine (**9**), or a D-phenylalanine (**11**) at position 2 produced little effect. Compound **11**, which has a D-phenylalanine, but no *N*-methyl moiety, exhibits 100-fold less potency than **10** (compare IC_{50} of **10** in Figure 7 to IC_{50} of **11** in HCT-116 = ~200 μM, HCT15 = ~300 μM).

Of all five positions, position 3 seems to have the greatest impact on cytoxicity (Figure 4). Placement of a single D-valine at position 3 (compound **13**) produces an extraordinarily potent compound against all colon cancer cell lines. An *N*-methyl D-valine at this position (**15**) also increases cytotoxicity relative to **1**. Yet, an *N*-methyl moiety alone (**12**) diminishes cytotoxicity relative to that of San A (**1**). Substitution of a relatively hydrophobic element such as D-phenylalanine (**18**) or a polar element such as D-serine (**17**) at position 3 diminishes the compounds activity relative to San A. The combination of a D-valine at position 3 and a D-leucine at 2 (**19**), a D-leucine at position 5 (**21**), or an *N*-methyl at position 5 (**22**) generates compounds that are less potent than compounds containing only D-valine at position 3 (**13**). However, an *N*-methyl D-valine at position 3, combined with a cyclohexyl moiety at position 4, generates a considerably potent compound (**16**). In contrast, an *N*-methyl D-valine at 3 combined with a D-leucine at 5 generates a nonpotent compound (**20**). Finally, two analogs containing a D-valine at 3 and a lysine-protected moiety at 4 (**24**) or an

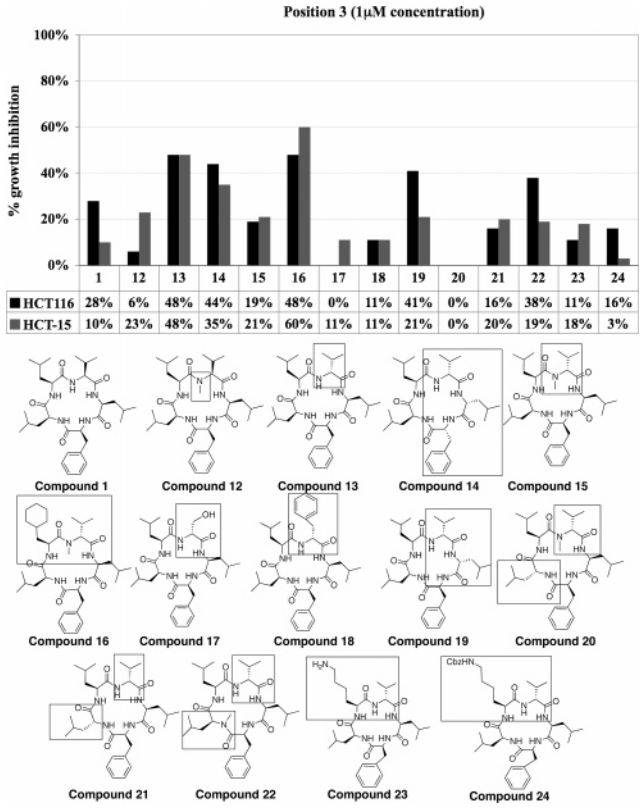

Figure 4. Compounds with changes at position 3. Each data point is an average of three wells run in two assays (error = ±5%).

unprotected lysine at 4 (**23**) both exhibit minimal cytotoxicity. Thus, compounds **13** and **16** show the greatest potency for drug-resistant colon cancer cancer cell lines in this series.

Changes at position 4 appear to have some impact on activity, where the inclusion of an *N*-methyl moiety (**26**) improves

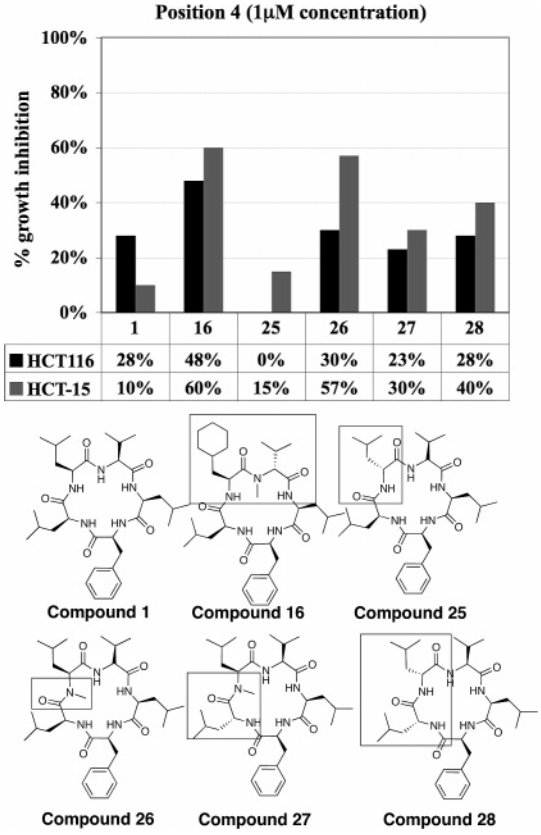

Figure 5. Compounds with changes at position 4. Each data point is an average of three wells run in two assays (error = ±5%).

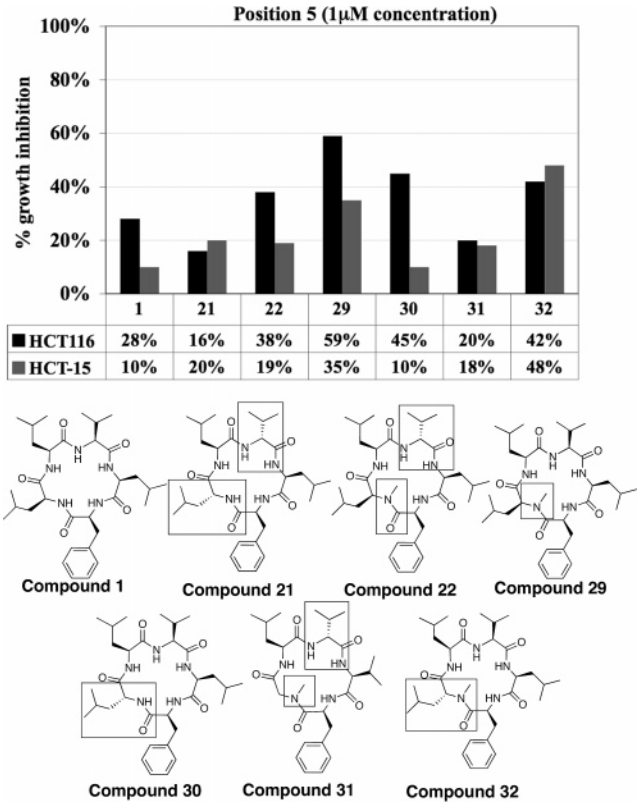

Figure 6. Compounds with changes at position 5. Each data point is an average of three wells run in two assays (error = ±5%).

cytotoxicity relative to San A (Figure 5). Compound **26** has an IC_{50} in the low micromolar range for HCT-116 and the high nanomolar range for HCT-15. In comparison to position 3,

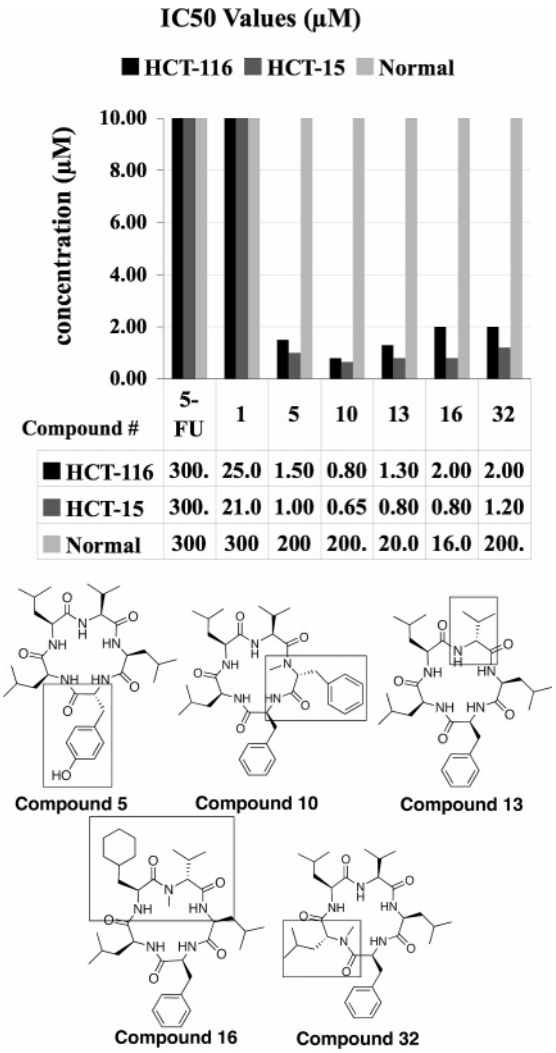

Figure 7. IC_{50} values of potent compounds. Each data point is an average of three wells run in two assays. Margin of error = ±5%. Normal cells are WS1.

neither the analog with a single D-aa at 4 (**25**) nor the combination of a D-aa in positions 4 and 5 (**28**) have improved cytotoxicity relative to San A. In addition, compound **27**, which contains an *N*-methyl moiety at 4 and a D-leucine at 5 demonstrates minimal cytotoxicity.

Position 5 appears to have a moderate impact on cytotoxicity where placement of a single *N*-methyl at 5 (**29**) manifests greater cytotoxicity than San A (Figure 6). A D-leucine at 5 does not dramatically improve cytotoxicity (**30**). In addition, compound **31**, which is a combination of potent compounds **13** and **29** is not very potent against colon cancers. Interestingly, placing an *N*-methyl-D-leucine at 5 (**32**) shows excellent potential as a lead compound. Yet a D-leucine or an *N*-methyl L-leucine at 5 combined with a D-valine at 3 (**21** and **22**, respectively) generates compounds that have lower cytotoxicity than San A.

The IC_{50} values for the five most potent compounds are shown in Figure 7. Compound **10** demonstrates nanomolar IC_{50} values for both drug-resistant colon cancers. Compound **10** is greater than 30-fold more potent than the natural product peptide **1**, possesses ~300-fold increased differential selectivity for cancer cell lines versus normal cell lines (WS1, skin fibroblasts) and is 450 times more active against colon cancer cell lines than compounds used clinically to treat these cancers (e.g., 5-FU).

After testing 32 compounds, we have come to the conclusion that not a single feature or position is critical to potency, rather as is typical in complex systems, there are several determining factors. The most important features to emerge from this SAR study include the incorporation of a D-tyrosine at position 1, an *N*-methyl D-phenylalanine at 2, a D-valine or an *N*-methyl D-valine at 3, an L-cyclohexyl moiety, and finally an *N*-methyl D-leucine at 5.

The inclusion of a single *N*-methyl D-amino acid (D-aa), as seen in the cases of **10**, **16**, and **32**, or an appropriately placed D-amino acid moiety, as seen with compounds **5** and **13**, appear to be crucial for potency. We hypothesize that a key connection between potency and structure involves constraining the macrocycle into its preferred and active conformation. Recent publications highlight that a single *N*-methyl D-aa is a key structural component required to maintain a dominant conformation in macrocycles with five amino acids.[9,10] Thus, the inclusion of a single *N*-methyl D-aa in our active structures most likely locks the ring into its low energy conformation and this conformation appropriately presents its side-chains to the biological target. Based on our current SAR we also believe that a single D-aa when appropriately placed will also lock the macrocycle into a low energy conformation. Molecular modeling show that a D-aa in positions 1 and 3 place the side chain in a pseudo equatorial position (whereas an L-aa in this position is in a pseudoaxial position), thus resulting in a low energy conformation. Thus, potency was achieved by incorporating a single *N*-methyl D-aa or an appropriately placed D-aa locking the macrocycle into a favorable binding conformation.

These data clearly show that significant progress has been made toward development of efficacious compounds against drug-resistant colon cancers. Specifically, we have identified five compounds that show 300-fold differential selectivity against colon cancer cell lines, have 450-fold greater cytotoxicity for drug-resistant colon cancer cell lines than the commonly used drug, 5-FU, and show potency on par with treatments used for other cancers. Importantly, we report here for the first time a nanomolar inhibitor of these drug-resistant cancer cell lines, compound **10**. These results clearly establish the San A scaffold as a promising structure for the development of new antitumor agents, and compounds incorporating the important structural features are being synthesized and will be reported in due course.

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Supporting Information Available: General cytotoxicity assay protocols are described. This material is available free of charge on the Internet at http://pubs.acs.org.

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