Synthesis and novel structure–activity relationships of potent sansalvamide A derivatives†

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Synthesis of twelve Sansalvamide A derivatives and their SAR against colon cancer (HT-29).

Sansalvamide A (San A) is a depsipeptide isolated from a marine fungus (*Fusarium ssp.*), which exhibits anti-tumor activity.[1–3] It is composed of four hydrophobic amino acids and one hydrophobic hydroxy-acid. Syntheses and evaluation of San A and the all-peptide analog have revealed that the peptide has greater potency against human colon carcinoma than the natural depsipeptide (cell line HCT-116).[4] Previous work by our group[5] and Silverman's group[6] reported several San A derivatives that demonstrated promising anti-cancer properties. To date, details about the mechanism of action of San A are incomplete.[6] We report here the synthesis of twelve San A derivatives, identify a key SAR (cell line HT-29), and demonstrate that this class of anti-tumor agent warrants further development.

We used a solution phase synthesis route because of the hydrophobic nature of the residues. Fig. 1 depicts the two fragments involved in our synthetic route. Our convergent approach[5] is amenable to easily inserting L- and D-amino acids in any position within San A. This route is also amenable to large-scale synthesis for extensive biological studies.

Synthesis of twelve San A derivatives were completed using amino acids shown (Fig. 2) and yielded structures shown in Fig. 3.[7]

The SAR was established using San A peptide [compound (**1**)] as a control. ^{3}H-thymidine uptake assays on HT-29 revealed four compounds, (**5**), (**6**), (**9**), and (**10**) had potent inhibitory activity (Fig. 4). These four compounds had IC_{50} values of 20 μg mL^{-1} (34 μM), 11 μg mL^{-1} (19 μM), 4.5 μg mL^{-1} (7.5 μM), and 20 μg mL^{-1} (33 μM) respectively (San A peptide IC_{50} = 45 μg mL^{-1}, 77 μM) and greater than 75% inhibitory activity. Compound (**9**) exhibited 90% growth inhibition and a 10 fold increased potency compared to San A peptide. It has been proposed by Silverman and co-workers[6] that the *N*-methyl moieties may be important for anti-tumor activity of San A derivatives in the HCT 116 cell line. Our data suggest that the *N*-methyl moiety does not play a role in potency against HT-29. Rather, potency is enhanced when compounds have a single L-amino acid exchanged to a structurally similar D-amino acid. Compounds (**5**) and (**10**) contain D-amino acids in position 2, and compounds (**6**) and (**9**) contain D-amino acids at position 3. Importantly, compounds (**2**) and (**9**) only differ by the exchange of an L-amino acid to a D-amino acid in position 3, yet (**9**) has a 40 fold increased potency over (**2**) ((**2**) has IC_{50} = 181 μg mL^{-1} [302 μM] and (**9**) IC_{50} = 4.5 μg mL^{-1} [7.5 μM]).

Further, comparison of compound (**1**) and compound (**2**), shows that the inclusion of the *N*-methyl group leads to a decrease in potency. Comparison of compound (**1**) and compound (**11**) shows the inclusion of the *N*-methyl group again leads to a decrease in potency. Finally, compounds (**3**) and (**12**), which both contain *N*-methyl D-amino acids seen in (**9**) and (**10**), are significantly less active than (**9**) or (**10**). It appears the SAR is related to a *single* D-amino acid in positions **2** or **3**, but not multiple D-amino acids within the San A structure. Interestingly,

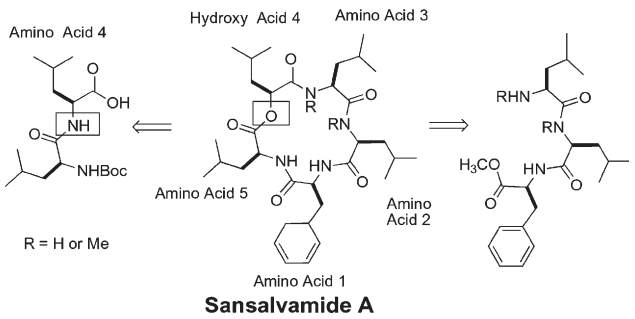

Fig. 1 Retrosynthetic approach of Sansalvamide A derivatives.

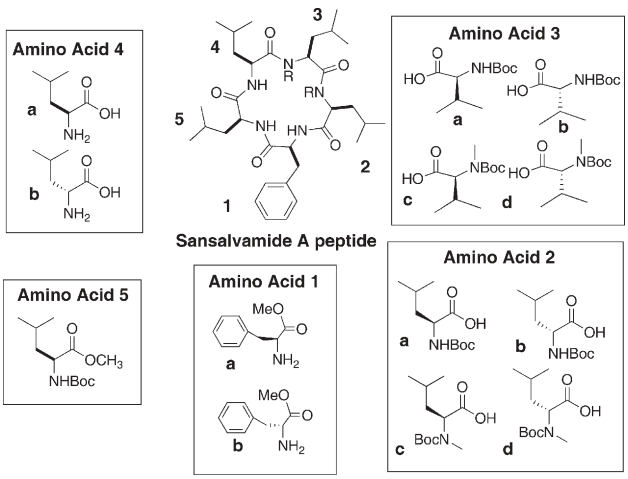

Fig. 2 Amino acids used in the synthesis of derivatives.

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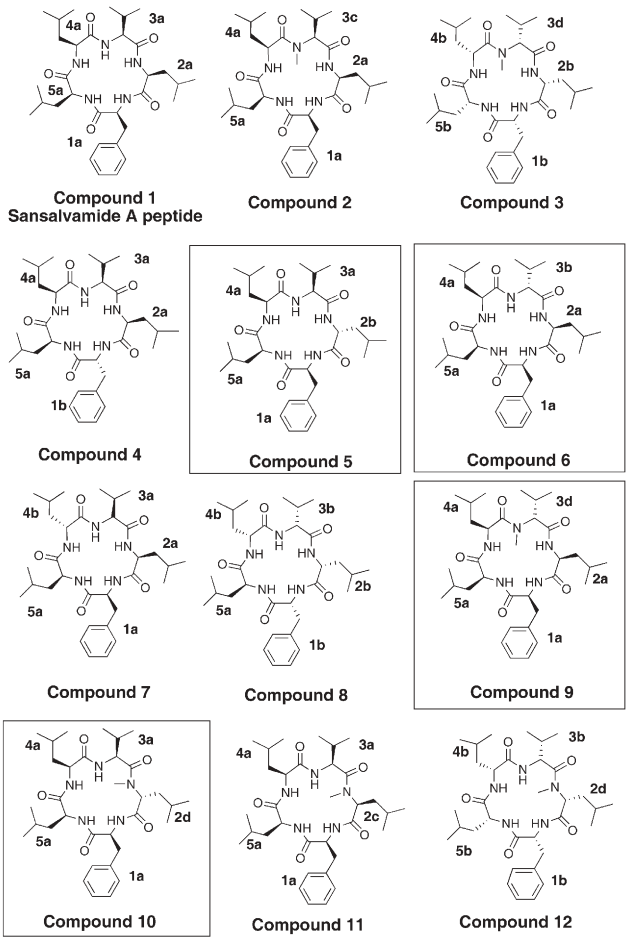

Fig. 3 Twelve compounds tested against colon cancer HT-29.

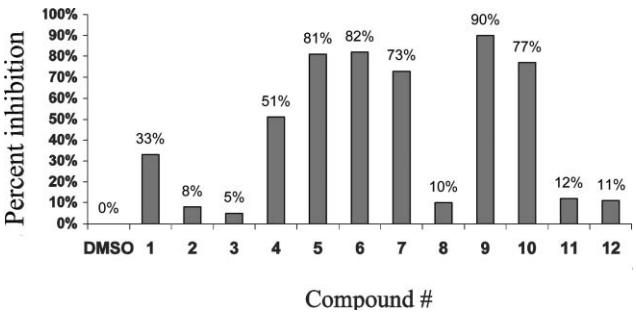

Fig. 4 Activity of twelve compounds in colon cancer (50 μM) detected in ^{3}H-thymidine uptake assays.

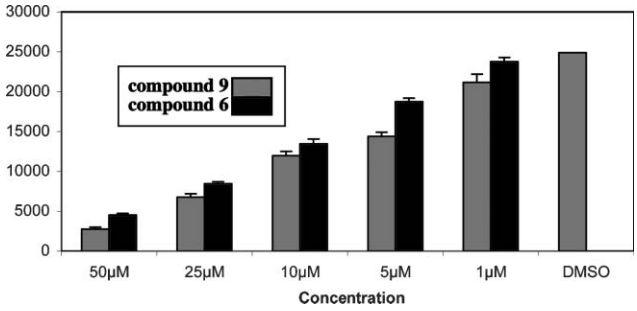

Fig. 5 Concentration dependent growth inhibition assays.

compounds (**4**) and (**7**) show enhanced potency compared to San A, and these compounds have positions 1 and 4 respectively exchanged from L to D. However, their potency is less than compounds (**5**), (**6**), (**9**), and (**10**).

Assaying the two most potent compounds at five concentrations demonstrated the linear effectiveness of these compounds against colon cancer HT-29 (Fig. 5).

In summary, four derivatives [(**5**), (**6**), (**9**), and (**10**)], exhibited potency comparable to that of a current drug on the market (Mitomycin C, IC_{50} = 5 μg mL^{-1}). This is the first example of such potent San A derivatives, and the first report of this novel SAR. The SAR seen in our data provides extraordinary promise in facilitating the design of new, potent San A analogues. Further investigation will be important to define the roles of D-amino acids and *N*-methyl amino acids. Additional assays of these compounds on other cancer cell lines are underway, and synthesis of a next generation utilizing the information described here are also in progress. These results will be reported in due course.

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