Conformational Analysis of Sansalvamide A: an Antineoplastic Group of Macrocyclic Peptides

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Abstract

Recent studies on the bio-potency of Sansalvamide A derivatives show promising properties against pancreatic, colon, breast, prostate, and melanoma cancers. Sansalvamide A (San A) is a marine fungal product that was discovered by William Fenical. Through structural manipulation of peptide derivatives based on structure-activity relationship (SAR) and 2-D NMR, valuable trends arise to provide a systematic means of controlling bio-potency. Through the use of various biological assays as well as computational resources it is possible to analyze the effect of such conformational changes and gain insight into the development of more potent compounds.

Using MacroModel within Maestro, we have validated the preferred conformation of many San A derivatives. Using Monte Carlo methods as well as conformational constraint and limiting electrostatic and steric qualifications, a pool of conformers was created. By arranging the lowest energy conformer within NAMFIS and using 2D NMR experiments NOESY and ROESY predictive studies of the proposed protein target of the derivatives are described. By performing pull-down assays we have determined that the protein target of these derivatives is Hsp90, and therefore computational validation combined with incorporation of the co-crystal structure of Hsp90-drug can be used to determine the active conformation of the molecule when bound to Hsp90. This crystalline matrix will then be analyzed using NAMFIS, a NMR based modeling program, to create a dynamical picture of the Sansalvamide A derivatives. Future potent derivatives can then be predicted using this hybrid computational/experimental approach.

History

- Discovered in 1999 by Professor W. Fenical *et. al Tetrahedron Lett.* V40, 2913-2916, 1999
- The natural product derivative: N-methyl Sansalvamide A discovered in 2000
- Synthesis of Sansalvamide A natural product by Professor R.B. Silverman in 2000
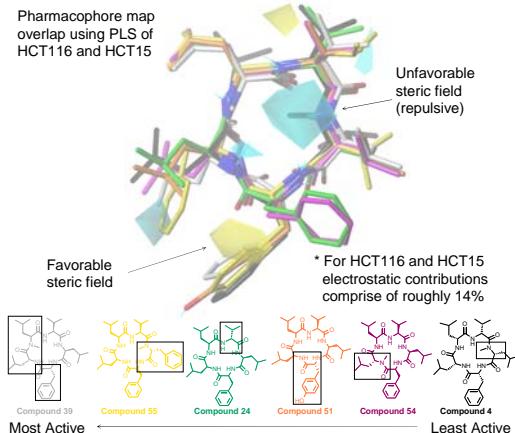- Synthesis of Sansalvamide A peptide by Professor R.B. Silverman in 2002

R = H or CH₃
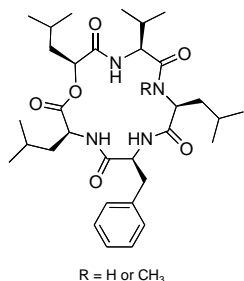
Synthesis and Evaluation
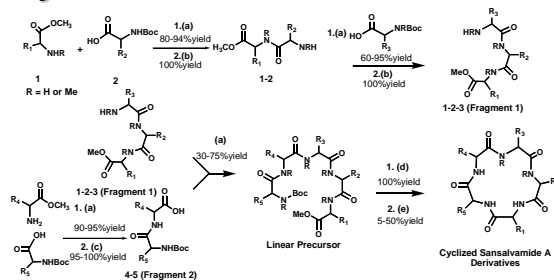
Conditions: a) coupling agent*, DIPEA (3 equiv), CH₂Cl₂ (0.1M), b)TFA (20%), Anisole (2 equiv), CH₂Cl₂, c) LiOH (8-12 equiv), MeOH d) HCl in THF (0.05M), Anisole (2 equiv), e) HATU (0.7 equiv), DEPBT (0.7 equiv), TBTU (0.7 equiv), DIPEA (6 equiv), THF:CH3CN:DCM (2:2:1) 0.007M.
* TBTU (1.2equiv), and/or HATU (0.75 equiv).
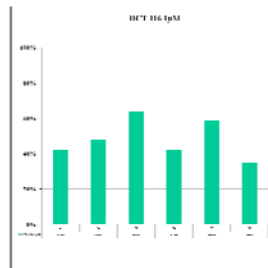
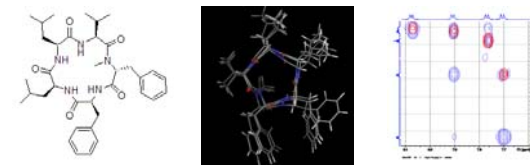Inhibitory concentration values for the San A derivatives shown with the pancreatic cancer cell line HCT-116. All values represented within +/- 5%.

NMR Analysis of Molecular Flexibility in Solution (NAMFIS)

The greatest issue in receiving a conformational picture of macrocyclic peptides by NMR is their tendency to dynamically fluctuate given the NMR solution. In order to correct this problem and create the most accurate picture of the molecules conformation as it presents itself to its protein target NAMFIS correlates experimental 2D NMR NOESY data along with co-crystal structures and computational searches. The program generates a throughput environment to experimentally validate computational resources.

Pharmacophore map overlay using PLS of HCT116 and HCT15

Unfavorable steric field (repulsive)

Favorable steric field

* For HCT116 and HCT15 electrostatic contributions comprise of roughly 14%

Compound 39   Compound 55   Compound 24   Compound 51   Compound 54   Compound 4

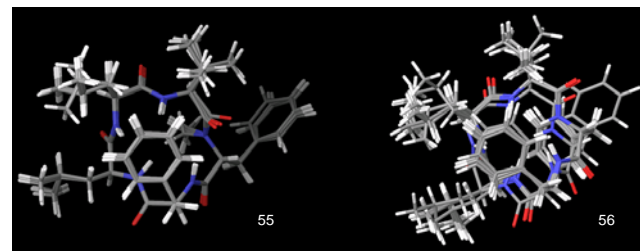Most Active ←   → Least Active

Comparative Molecular Field Analysis (CoMFA)

CoMFA is a 3D quantitative structure-activity relationship technique used in predicting key molecular fields within a molecule. A pharmacophore map is generated after steric and electrostatic fields are calculated for key molecules by interacting a probe atom with a series of points surrounding the 3D molecule. These energy terms are compared with biological information using partial least squares (PLS) with cross-validation. Within the conformation of San A it is not only important to note the overall orientation of the molecule, but the molecular fields generated as a result of the conformation. The molecular field of interest is the steric field as it will determine the site available for binding with the protein target.

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Conformational Searches: Maestro

Using Schrodinger's MacroModel within the Maestro console the following compounds were analyzed. Using Monte Carlo methods as well as conformational constraint and limiting electrostatic and steric qualifications, a pool of conformers was created. By superimposing the lowest energy conformers on the peptide backbone an average picture of the molecules conformation was analyzed. The result of the searches was the discovery of key residues and functional groups that generate a more rigid conformer, thus resulting in a dynamic picture of the preferred conformation for the protein target.

Acknowledgments

1) Thomas Styers, Ahmet Kekec, Rodrigo Rodriguez, Joe Brown, Julia Cajia, Chris L. Carroll, Ricardo Corral, Jennifer Johnston, Irene Medina, Po-Shen Pan, Emily Parry, Stephanie Lapera, *Bioorganic & Medicinal Chemistry Letters,*V14 P5625-5631, **2006**
2) Rodrigo Rodriguez, Po-Shen Pan, Chung-Mao Pan, Suchitra Ravula, Erin Singh, Stephanie Lapera, Thomas J. Styers, Joseph D. Brown, Julia Cajica, Emily Parry, and Shelli R. McAlpine* *J. Org. Chem. 72(6), 1980-2002,* **2007**
3) Mohamadi, F., Richard, N. G. J., Guida, W. C., Liskamp R., Lipton, M., Caufield, C., Chang, G., Hendrickson, T., Still, W. C., "MacroModel - an Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics," *J. Comput. Chem.*, (1990) **11**, 440.
4) Cicero DO, Barbato G, Bazzo R . *J Am Chem Soc* 1995, **117:**1027-1033.
5) Cramer, R.D., III, D.E. Patterson, and J.D. Bunce,. *J. Am. Chem. Soc.*, 1988. **110(18):** p. 5959-67.