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Synthesis of Axinastatins 2 - 5

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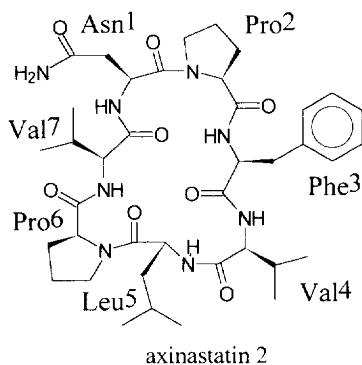
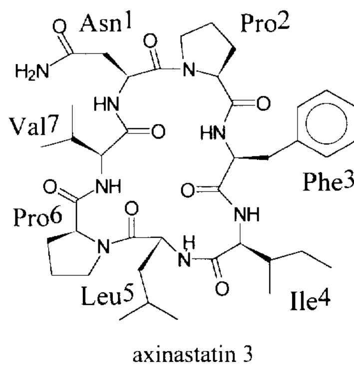
Abstract: The cytotoxic cyclic peptides axinastatin 2, 3, 4 and 5, isolated from the ocean marine sponges *Axinella sp.* and *Axinella cf. carteri*, were synthesized and characterized by FAB-MS and homo- and heteronuclear NMR spectroscopy. The synthetic compounds proved to be identical with the natural products. The cyclization yield of axinastatin 5 could be increased using structural information of a conformationaly comparable compound. In contrast to the published biological activity very low activities were found for all compounds. Copyright © 1996 Elsevier Science Ltd

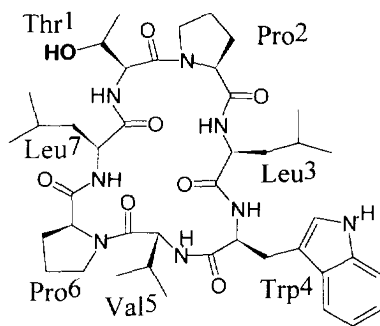
During the last years, numerous natural products with various biological activities have been isolated from lower marine animals and plants.[1] Besides new antibiotics, a large number of cyclic peptides with cytostatic properties such as hymenistatin,[2] stylostatin,[3] axinastatin 1,[4] and the group of discokinolides[5] and theonellamides[6] were obtained. From the Republic of Palau marine sponge *Axinella sp.* and the Republic of the Comores marine sponge *Axinella cf. carteri* the cyclic heptapeptides axinastatin 2, 3,[7] and 4,[8] and the cyclic octapeptide axinastatin 5[9] were isolated by Pettit and coworkers in 10^{-7} % to 10^{-6} % yield.



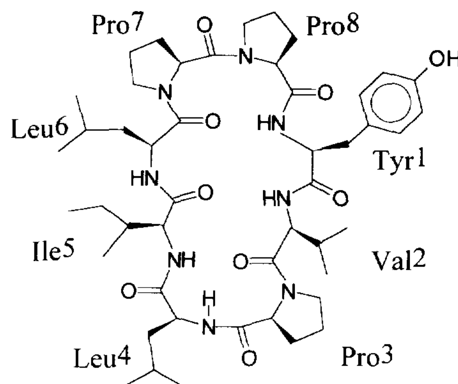
axinastatin 2



axinastatin 3



axinastatin 4



axinastatin 5

The primary structure of the compounds were determined as cyclo(-Asn^1-Pro^2-Phe^3-Val^4-Leu^5-Pro^6-Val^7-) for axinastatin 2, cyclo(-Asn^1-Pro^2-Phe^3-Ile^4-Leu^5-Pro^6-Val^7-) for axinastatin 3, cyclo(-Thr^1-Pro^2-Leu^3-Trp^4-Val^5-Pro^6-Leu^7-) for axinastatin 4 and cyclo(-Tyr^1-Val^2-Pro^3-Leu^4-Ile^5-Leu^6-Pro^7-Pro^8-) for axinastatin 5 by chemical methods as well as NMR and MS experiments.[7,8,9] Showing a strong activity against several human cancer cell lines with GI_{50} values between 3.3 and 0.0072 μg/ml, these cyclic peptides seem to be promising compounds for structure-activity-relationship studies and are predestinated for the design of new lead structures and agents against cancer.

In the course of our investigations of peptidic anticancer agents,[10,11] axinastatin 2, 3, 4 and 5 were synthesized to verify the proposed primary structure of the isolated compounds and provide sufficient amounts of the biological active compounds for further structural and pharmaceutical investigations.

The synthesis of axinastatin 2, 3, 4 and 5 was carried out by cyclization of linear, side-chain protected peptide building blocks. The linear peptides were assembled in 77% to 83% yield by solid-phase synthesis[12] using the Fmoc-strategy,[13] o-chlorotrityl chloride-resin,[14] and TBTU/HOBt[15] as coupling reagent. Threonine and tyrosine were protected as t-butyl ether; asparagine was used tritylated. Tryptophan was used without side chain protection.

Generally the cyclization is considered to be the limiting step in the synthesis of cyclic all-L-peptides. The yields strongly depend on the cyclization-site.[16] The cyclization was carried out at high dilution ($1.25*10^{-3}$M) in DMF using DPPA/$NaHCO_3$[17] as cyclization reagent. Cyclization between Pro^6 and Val^7 provided the side chain protected axinastatin 2 in 3% and could be improved by cyclization between Val^7 and Asn^1 to 24% yield. Analogous experiments afforded the side chain protected axinastatin 3 in 1% and 23% yield, respectively. The cyclization of axinastatin 4 was successful between Leu^3 and Trp^4 with 19 % cyclization yield.

Our recent cyclization experiments suggest a successful cyclization of all-L-peptides between the *i+2* and *i+3* position of a β-turn in the final cyclic structure. The structure[11] of hymenistatin (cyclo(-Tyr^1-Val^2-Pro^3-Leu^4-Ile^5-Ile^6-Pro^7-Pro^8-)) has a βI-turn in $CHCl_3$ and a βII-turn in DMSO about Pro^3 and Leu^4. The exchange of only one amino acid (Leu^6 vs. Ile^6) in axinastatin 5 should have little influence on the secondary structure elements. Indeed, cyclization between Leu^4 and Ile^5, the presumed *i+2* and *i+3* position provided the side chain protected axinastatin 5 in moderate yields of 28%.

The protecting groups were removed from the cyclized peptides with TFA containing ethanedithiol/H_2O as scavenger. Purification by RP-HPLC (standard acetonitrile/water gradient system) afforded axinastatin 2 and 3 in 11%, axinastatin 4 in 7% and axinastatin 5 in 14% yield.

The synthetic compounds were characterized by FAB-MS (axinastatin 2: m/z: 767 $[M+H^+]$; axinastatin 3: m/z: 781 $[M+H^+]$; axinastatin 4: m/z: 808 $[M+H^+]$; axinastatin 5: m/z: 893 $[M+H^+]$) and two-dimensional NMR spectroscopy (TOCSY, PE-COSY, ROESY, HMQC-COSY, HMBC). The ^1H and ^{13}C chemical shifts were identical to those described for the natural product.[18,19] Especially ^{13}C shift data strongly depend on conformational (and hence also on constitutional) changes. Their identity therefore can serve as proof for structural identity.

However, contrary to the published biological activity of the natural products our first results in different human cancer cell lines determine the group of axinastatins to be inactive or of low activity. This could be explained by small amounts of additional very active compounds in the natural product.

Conformational analysis of axinastatin 2, 3, 4 and 5 by high-field NMR spectroscopy in combination with MD calculations in explicit solvents will be published separately with detailed biological results.

ACKNOWLEDGEMENTS

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