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SYNTHESIS OF NAPAVIN, A NEW PHOTOREACTIVE DERIVATIVE OF VINBLASTINE

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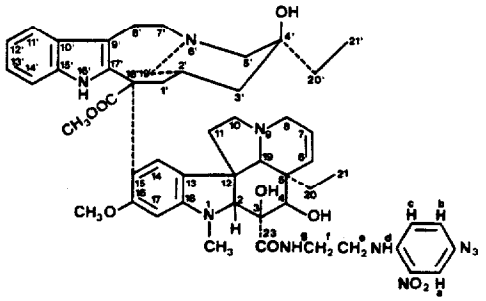
Summary: The synthesis of 3-[[[2-amino(4-azido-2-nitrophenyl)ethyl]amino]carbonyl]-O^4-deacetyl-3-de-(methoxycarbonyl)-vincaleukoblastine (napavin) from vinblastine is described. This new photoreactive cytostatic substance overcomes multidrug resistance of tumor cells.

The vinca alkaloid vinblastine is a clinically successful cytostatic drug which acts by binding noncovalently to the intracellular protein tubulin, preventing it from assembly into the mitotic spindle that is essential for cell proliferation. However, tumors tend to become resistant to it during treatment. In a frequent form of multidrug-resistance, an outward transport protein in the cell membrane rapidly eliminates a variety of chemically unrelated drugs from cells, rendering them insensitive to high extracellular drug concentrations. We reasoned that intracellular formation of covalent bonds between vinblastine and target proteins would prevent the alkaloid being eliminated, increase the half life of its action, and possibly block the outward transport mechanism. We decided to synthesize a derivative that can be activated to form covalent bonds upon irradiation with light at a wavelength and energy level that would neither damage the alkaloid, DNA or other essential cellular components. Here, we describe the synthesis and characterization of a compound, napavin that has the desired properties.[1,2] It is activated by an argon laser at 457 nm.

Synthesis. Napavin was synthesized by coupling 4-(2-aminoethyl)amino-3-nitro-phenylazide (napa) to deacetylvinblastine acid azide. The procedure for the preparation of the latter compound from vinblastine free base was based on that of Barnett et al. [3], and Rao et al. [4], respectively. In short, the free base was heated at 60 °C with anhydrous hydrazine under an argon atmosphere. Progress of hydrazinolysis was monitored by thin layer chromatography on silica gel sheets using CH_2Cl_2 / CH_3OH, 1: 5 (solvent A) and methanol/ acetone, 1: 1 (solvent B). Deacetylvinblastine monohydrazide was extracted with CH_2Cl_2, washed with saturated NaCl and dried over Na_2SO_4. The product was crystallized from CH_2Cl_2 at 4 °C. Deacetylvinblastine acid azide was prepared by reducing the monohydrazide at -10 °C in a solution of methanol/1 M HCl , 3:1 with $NaNO_2$. The pH was adjusted to 8.8 with saturated $NaHCO_3/Na_2CO_3$ and the acid azide was extracted immediately with CH_2Cl_2, washed with saturated NaCl, and dried over Na_2SO_4. R_f 0.68 (solvent A), 0.40 (solvent B). Yield 60%, based on vinblastine sulfate.

4-(2-Aminoethyl)amino-3-nitro-phenylazide (napa) was synthesized in the dark by adding with stirring at room temperature, 455 mg (2.5 mmol) of 4-fluoro-3-nitrophenylazide dissolved in 5 ml of CH_2Cl_2 to a mixture of 1.6 ml (24 mmol) ethylendiamine and 3.4 ml CH_2Cl_2. After 30 min the solution was diluted with 10 ml of CH_2Cl_2 and washed twice with 15 ml of H_2O. The organic phase was evaporated to dryness and napa was crytallized from 30 ml of ether/hexane (1:1) at 4 °C under N_2. Yield, 388 mg (1.75 mmol), 70% based on fluoronitrophenylazide. R_f 0.28 (solvent A), 0.50 (solvent B).

Napavin was synthesized in the dark by linking napa to the deacetylvinblastine acid azide-23-oyl moiety through an amide bond. The acid azide (155 mg, 0.20 mmol) in 4 ml CH_2Cl_2 was added to 111 mg (0.50 mmol) napa dissolved in 6 ml CH_2Cl_2 and the mixture was stirred for 40 h at room temperature. The progress of the reaction was monitored by HPLC. Solvent was evaporated under argon and the product dissolved in 80% methanol. Separation of napavin from the byproducts was achieved by reversed phase high pressure liquid chromatography on a C18 preparative column (Waters μBondapak, 19 x 150 mm), using a linear gradient from 40% methanol in 6 mM potassium phosphate, pH 4.4, to 90% methanol in 1 mM potassiom phosphate. For removal of salt, napavin was dissolved in methanol. Yield, 81 mg (0.085 mmol) 26%, based on vinblastine sulfate. Rf 0.50 (solvent A), 0.52 (solvent B). Napavin was stored dry at -70 °C and protected from light.



3-[[[2-amino(4-azido-2-nitrophenyl)ethyl]amino]carbonyl]-O^4-deacetyl-3-de(methoxycarbonyl)-vincaleukoblastine (NAPAVIN)

Selected physical data. UV/VIS: broad λ_{max} in the 450 - 490 nm range (aminonitrophenyl azide) (ε_{455} = 5,200 M^{-1} cm^{-1}), λ_{max} 260 nm (nitrophenylazide and vinca moiety). IR (KBr): ν = 2130 cm^{-1} (N_3), ^{1}H-NMR (500 MHz, int. TMS, [D_6]-DMSO): devoid of δ 1.98 (C^4-$OCOCH_3$) and δ 3.64 (C^3-CO_2CH_3) in vinblastine [5], whereas δ 3.72 (C^{16}-OCH_3), δ 3.56 ($C^{18'}$-CO_2CH_3) and δ 7.29, 7.38 and 7.72 (phenyl) were present. MS (L-SIMS): m/z $[M+1]^+$ 959 and 931 (compound minus N_2). The ^{1}H-NMR signals and m/z peaks of all intermediates were in agreement with the designations given.

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