Supplementary Material

Synthesis of lasalocid-based bioconjugates and evaluation of their anticancer activity

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General procedures

All reagents and solvents were obtained from Merck or Trimen Chemicals S.A. (Poland), and were used as received without further purification. CDCl₃, CD₂Cl₂ and CD₃CN spectral grade solvent was stored over 3Å molecular sieves for several days. All manipulations were carried out under nitrogen atmosphere in oven-dried glassware. Reaction mixtures were stirred using teflon-coated magnetic stir bars. Reaction mixtures were monitored by thin layer chromatography (TLC) using aluminium-backed plates (Merck 60F₂₅₄). TLC plates were visualized by UV-light (254 nm), after treated with phosphomolybdic acid (PMA, 5% in absolute EtOH) and gentle heating. Products of the reactions were purified using CombiFlash Rf⁺ Lumen Flash Chromatography System (Teledyne Isco) with integrated ELS and UV detectors. All solvents used in flash chromatography were of HPLC grade (Merck), and were used as received. Solvents were removed using a rotary evaporator.

NMR spectra were recorded on a Varian 400 (¹H NMR at 400 MHz, ¹³C NMR at 101 MHz, ¹⁹F NMR at 282 MHz, and ³¹P NMR at 162 MHz) magnetic resonance spectrometer. ¹H NMR spectra are reported in chemical shifts downfield from TMS using the respective residual solvent peak as internal standard (CDCl₃ δ 7.26 ppm, CD₂Cl₂ δ 5.32 ppm, or CD₃CN δ 1.94 ppm). ¹H NMR spectra are reported as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets, td = triplet of doublets, pd = pentet of doublets, ddd = doublet of doublet of doublets, ddt = doublet of doublet of triplets, tdd = triplet of doublet of doublets, m = multiplet), coupling constant(s) in Hz, and integration. Significant peaks are reported within the overlapping ~2.10–0.60 ppm region of the ¹H NMR spectra. ¹³C NMR spectra are reported in chemical shifts downfield from TMS using the respective residual solvent peak as internal standard (CDCl₃ δ 77.16 ppm, CD₂Cl₂ δ 53.84 ppm, or CD₃CN δ 1.32 ppm and 118.26 ppm). ¹⁹F NMR spectra are reported in chemical shifts upfield from TMS using CFCl₃ as internal standard. Line broadening parameters were 0.5 or 1.0 Hz, while the error of chemical shift value was 0.1 ppm.

Infrared spectra in the mid infrared region were recorded for KBr tablets on an IFS 113v FT-IR spectrophotometer (Bruker) equipped with a DTGS detector, and are reported as follows: wavenumbers (cm⁻¹), description (w = weak, m = medium, s = strong, br = broad). The spectra were taken at a resolution 2 cm⁻¹, NSS = 64. The Happ-Genzel apodization function was used.

Electrospray ionization (ESI) mass spectra were recorded on a Waters/Micromass ZQ mass spectrometer (Waters Alliance) equipped with a Harvard syringe pump. Samples were prepared in dry acetonitrile, and were infused into the ESI source using a Harvard pump at a flow rate of 20 mL min⁻¹. The ESI source potentials were: capillary 3 kV, lens 0.5 kV, and extractor 4 V. Standard ESI mass spectra were recorded at the cone voltages of 10 and 30 V. The source temperature was 120 °C and the desolvation temperature was 300 °C. Nitrogen was used as the nebulizing and desolvation gas at flow-rates of 100 dm³ h⁻¹. Mass spectra were acquired in the positive ion detection mode with unit mass resolution at a step of 1 m/z unit. The mass range for ESI experiments was from m/z = 300 to m/z = 1100, or m/z = 300 to m/z = 1300. High-resolution mass spectra (HRMS) were recorded on a QTOF mass spectrometer (Impact HD, Bruker Daltonics).
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