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Spectroscopic and structural studies of a new *para*-iodo-*N*-benzyl amide of salinomycin

Michał Antoszczak [a, *], Jan Janczak [b], Jacek Rutkowski [a], Bogumił Brzezinski [a], Adam Huczyński [a]

[a] Faculty of Chemistry, Adam Mickiewicz University, Umultowska 89b, 61-614, Poznań, Poland
[b] Institute of Low Temperature and Structure Research, Polish Academy of Sciences, PO Box 1410, 50-950, Wrocław, Poland

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ABSTRACT

A new *para*-iodo-*N*-benzyl amide of salinomycin was synthesized and characterized by NMR, FT-IR, DFT, single crystal X-ray diffraction and theoretical methods. The results obtained for the crystal, in solution and in gas phase provided evidence of pseudo-cyclic structure of this compound stabilized by intramolecular hydrogen bonds. It was shown that the compound studied forms stable 1:1 complexes with monovalent (Li^+, Na^+, K^+, Rb^+ and Cs^+) and divalent (Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}) cations demonstrating that the chemical modification of salinomycin carboxyl group considerably changes the ionophoretic properties of this antibiotic. For the first time, the ESI MS fragmentations of the complex of *para*-iodo-*N*-benzyl amide of salinomycin with Na^+ are also discussed in details.

1. Introduction

Salinomycin (**SAL**, Scheme 1) belongs to the group of natural polyether antibiotics (ionophores) and for decades it has been widely used in veterinary medicine as non-hormonal and growth-promoting agent. Biological activity of **SAL** is clearly dependent on its ability to form complexes with metal cations, especially Na^+ and K^+, and transport them across the cell membranes [1]. Recently, it has been also proved that **SAL** is able to form stable complexes with organic amines [2].

SAL was isolated for the first time in 1974 from the cultured broth of *Streptomyces albus* [3]. A *para*-iodophenacyl ester obtained one year later was the first successfully solved crystalline derivative of **SAL**, which helped to establish the entire molecular structure and the absolute configuration of this antibiotic [4].

In 2009 Gupta and co-workers [5] documented that **SAL** was very effective in the fight against breast cancer stem cells (CSCs) against about 16.000 substances used in these studies. It is worth mentioning that **SAL** was ~100-fold more effective than the commonly used cytostatic drug *Paclitaxel* [5]. In subsequent years **SAL** was also demonstrated to induce antiproliferating process of many human cancer cells, including prostate, lung, ovarian and leukaemic cancer cells [6,7]. In 2012 Naujokat and Steinhart [8] used **SAL** for the first time in cancer therapy of humans. The clinical trial was performed on a small group of patients with invasive carcinoma of the head, neck, breast and ovary [8].

Due to a broad spectrum of interesting biological and pharmacological properties exhibited by **SAL**, the natural direction of research is its chemical modification, which can lead to obtain unique derivatives with significantly better biological activity and lower toxicity than those of the unmodified antibiotic. The syntheses and biological activity of different **SAL** derivatives have been described, including its amides, esters, chemo- and regioselective modified analogues [9–16] as well as natural product conjugates [17–20].

Evaluation of the results of biological activity tests clearly proved that some of **SAL** C(1) amides and esters show improved activity against multi-drug resistant (MDR) cancer cells and good selectivity of action [9–11]. On the other hand, it has been shown that C(20) esters, carbonates and carbamates of **SAL** are characterized by IC_{50} values down to one fifth that of **SAL** [12], and the most active member in each class of C(20) *O*-acylated analogues

* Corresponding author.
E-mail address: michant@amu.edu.pl (M. Antoszczak).

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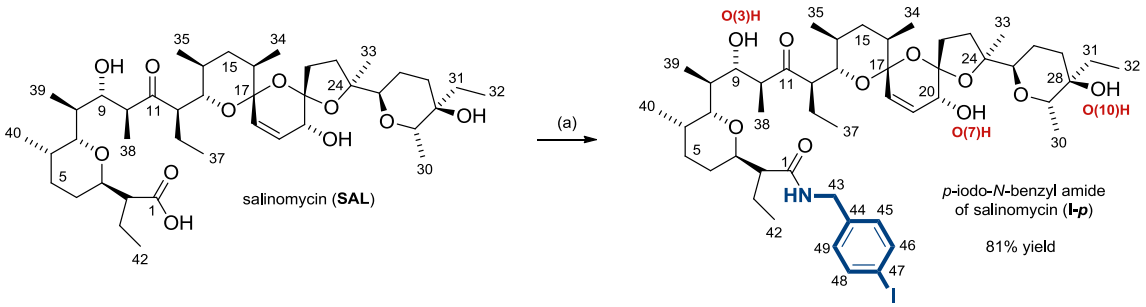

Scheme 1. Synthesis of the *para*-iodo-*N*-benzyl amide of **SAL** (**I-*p* amide**) and the numbering of the carbon atoms and hydroxyl groups. *Reagents and conditions*: (a) **SAL** (1.0 eq), DCC (1.2 eq), HOBt (0.5 eq), *para*-iodobenzylamine hydrochloride (2.0 eq), Et_3N (2.0 eq), DMF, 0 °C to rt, 24 h.

exhibits improved activity against CSCs across several assays even at nM concentrations [21].

Results of earlier studies mentioned above encouraged us to synthesize a new *para*-iodo-*N*-benzyl amide of salinomycin (**I-*p* amide**, Scheme 1). Because the biological activity of **SAL** and its derivatives is strictly connected with their characteristic pseudo-cyclic structure, we focused here on the detailed spectroscopic, structural and spectrometric studies of **I-*p* amide** using various methods, including spectroscopic (FT-IR, ^1H, ^{13}C, 2D NMR), spectrometric (ESI MS), DFT and single crystal X-ray diffraction ones.

2. Experimental section

2.1. General

Salinomycin (**SAL**) was prepared conveniently by isolation of its sodium salt from commercially available veterinary premix SACOX® following acidic extraction, using the procedure described by us previously [9–11].

N,*N*′-dicyclohexylcarbodiimide (DCC), *para*-iodobenzylamine hydrochloride and the applied solvents were commercial products of Sigma-Aldrich or Fluka, and were used without any further purification, 1-hydroxybenzotriazole hydrate (HOBt) was obtained from AK Scientific. Dichloromethane-d_2 spectral grade solvent (Sigma-Aldrich) was stored over 3 Å molecular sieves for several days. Triethylamine (≥99.5%), dichloromethane (for FT-IR spectroscopy) and acetonitrile (HPLC grade, ≥99.9%) were purchased from Fluka or Sigma-Aldrich, and were used as received without further purification. Handling of the compounds was performed in a carefully dried CO_2-free glove box.

2.2. Synthesis of para-iodo-N-benzyl amide of salinomycin (*I-p amide*)

To a mixture of **SAL** (500 mg, 0.66 mmol) in DMF (15 mL) the following compounds were added: DCC (206 mg, 1.00 mmol), HOBt (45 mg, 0.33 mmol), *para*-iodobenzylamine hydrochloride (310 mg, 1.33 mmol) and triethylamine (134 mg, 1.33 mmol). Firstly, the mixture was stirred at a temperature below 0 °C for 6 h, and then for further 18 h at room temperature. The solvent was subsequently evaporated, under reduced pressure, to dryness. The residue was suspended in methylene chloride (10 mL) and filtered off. The filtrate was evaporated under reduced pressure and the residue was purified chromatographically (dichloromethane/THF 100:3 as mobile phase) on silica gel (Fluka type 60) to give *para*-iodo-*N*-benzyl amide of salinomycin (**I-*p* amide**) (521 mg, 81% yield) as a white amorphous solid.

The parallelepiped colorless X-ray quality single crystals of the **I-*p* amide** were grown by slow evaporation from acetonitrile solution (151 mg, 29% yield). Mp = 215–218 °C. ^1H NMR (600 MHz, CD_2Cl_2) δ (ppm) 7.61–7.58 (*m*, 2H), 7.15–7.10 (*m*, 2H), 7.06 (*t*, J = 5.8 Hz, 1H), 6.09 (*dd*, J = 10.8, 1.5 Hz, 1H), 5.96 (*dd*, J = 10.7, 1.0 Hz, 1H), 4.93 (*dd*, J = 15.3, 7.0 Hz, 1H), 4.57 (*dd*, J = 15.4, 4.5 Hz, 1H), 4.26–4.17 (*m*, 1H), 4.03–3.98 (*m*, 2H), 3.86 (*d*, J = 7.7 Hz, 1H), 3.76 (*dd*, J = 13.4, 6.6 Hz, 1H), 3.71 (*ddd*, J = 9.1, 7.5, 1.7 Hz, 2H), 3.45 (*dd*, J = 12.1, 2.0 Hz, 1H), 2.96 (*td*, J = 14.9, 7.3 Hz, 1H), 2.76–2.66 (*m*, 2H), 2.56 (*dt*, J = 9.0, 2.7 Hz, 1H), 2.43 (*s*, 1H), 2.30 (*dt*, J = 12.4, 10.0 Hz, 1H), 2.09 (*ddd*, J = 11.9, 9.9, 2.1 Hz, 1H), 1.97–0.67 (*m*, 40H), 1.20 (*d*, J = 6.8 Hz, 3H), 1.08 (*s*, 3H), 0.77 (*d*, J = 6.8 Hz, 3H), 0.71 (*d*, J = 6.7 Hz, 3H). ^{13}C NMR (151 MHz, CD_2Cl_2) δ (ppm) 213.8, 176.0, 140.4, 137.5, 133.6, 130.0, 120.8, 106.7, 99.1, 92.0, 89.0, 79.9, 77.1, 75.9, 74.6, 71.5, 71.0, 69.6, 67.4, 54.1, 48.7, 46.9, 42.6, 40.7, 38.7, 37.1, 36.7, 33.1, 31.2, 30.4, 29.5, 28.6, 27.0, 26.2, 22.2, 22.0, 20.8, 18.1, 17.4, 15.7, 14.9, 14.7, 14.2, 12.3, 11.6, 8.4, 6.4.

2.3. X-ray measurements

The single crystal X-ray diffraction measurements of **I-*p* amide** were performed at 295 K on a four-circle KUMA KM4 diffractometer equipped with two-dimensional CCD area detector. Graphite monochromatized MoKα radiation (λ = 0.71073 Å) and ω-scan technique ($\Delta\omega$ = 1°) were used for data collection. The data collection and reduction along with absorption correction were performed using CrysAlis software package [22]. The structure was solved by direct methods using SHELXS-97 [23], which revealed the positions of almost all non-hydrogen atoms. The remaining atoms were located from subsequent difference Fourier syntheses. The structure was refined by full-matrix least-squares with SHELXL-2014/7 [24] with the anisotropic thermal displacement parameters. Visualization of the structure was made using Diamond 3.0 program [25]. The details of the data collection parameters, crystallographic data and final agreement parameters are collected in Table 1.

Details of the data collection and refinement, fractional atomic coordinates, anisotropic displacement parameters as well as full list of bond lengths and angles in CIF format have been deposited at the Cambridge Crystallographic Data Centre, No. CCDC 1493355. Copies of this information may be obtained free of charge from the CCDC, 12 Union Road, Cambridge, CB2 1EZ, United Kingdom (phone: +44 1223 336 408, fax: +44 1223 336 033, e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

2.4. Theoretical calculations

Ab-initio molecular orbital calculations with full geometry optimization of **I-*p* amide** were performed with the Gaussian03 program package [26]. All calculations were carried out with the density functional three-parameter hybrid (B3LYP) methods [27,28] assuming the geometry resulting from the X-ray diffraction study as the starting structure. As convergence criterions the threshold