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Preliminary communication

# Synthesis and biological activity of salinomycin conjugates with floxuridine

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ABSTRACT

As part of our program to develop anticancer agents, we have synthesized new compounds, which are conjugates between well-known anticancer drug, floxuridine and salinomycin which is able to selectivity kill cancer stem cells. The conjugates were obtained in two ways *i.e.* by copper(I) catalysed click Huisgen cycloaddition reaction performed between 3′-azido-2′,3′-dideoxy-5-fluorouridine and salinomycin propargyl amide, and by the ester synthesis starting from salinomycin and floxuridine under mild condition. The compounds obtained were characterized by spectroscopic methods and evaluated for their *in vitro* cytotoxicity against seven human cancer cell lines as well as antibacterial activity against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE). The conjugate obtained by esterification reaction showed a significantly higher antiproliferative activity against the drug-resistant cancer cells and lower toxicity than those of salinomycin and floxuridine towards normal cells, as well as standard anticancer drugs, such as cisplatin and doxorubicin. The conjugate compound revealed also moderate activity against MRSA and MRSE bacterial strains. Very high activity of floxuridine and 5-fluorouracil against MRSA and MRSE has been also observed.



## 1. Introduction

One of the current concepts in the anticancer drug design and development is synthesis of new hybrid compounds (molecular hybridization/bioconjugation) of improved affinity and efficacy relative to those of the parent drugs [1,2]. Pharmacophore conjugation is believed to be analogous to the conventional combination therapy, but with the two drugs covalently linked and available as a single entity [3].

The development of nucleoside analogues for medicinal use has significant impact on clinical chemotherapy as applied to antiviral and anticancer treatment [4–8]. Since its first discovery in 1957, 5-fluorouracil (**FUra**) has been a well-known anti-tumour agent used in the treatment of several neoplastic diseases, such as colon or breast cancers [9,10]. **FUra** is an anticancer drug with tremendous clinical potential, but has had limited efficacy due to its high toxicity [11,12]. Numerous **FUra** derivatives have been developed up to now. One of the best known **FUra** derivative is 5-fluoro-2′-deoxyuridine (**FdU**, Floxuridine), which is known for its high anti-tumour activity against cancer metastases [13,14]. **FdU** and other nucleoside analogues have been extensively used in the treatment of a variety of cancers over the last 40 years, and their mechanisms of action are well understood. **FdU** has been used to treat human solid tumours. However, **FdU** exhibited various side effects in the clinical treatment and its therapeutic effect was limited by the efficiency of cellular uptake and bioavailability of the drug [15]. In order to overcome these limitations various synthetic approaches have been proposed to improve its physicochemical properties and reduce its toxicity [16].

On the other hand, it is well known that natural products have provided some of the most effective drugs for the treatment of cancer, including paclitaxel, vinblastine, vincristine and doxorubicin [17,18]. Very interesting and time-consuming studies were performed in 2009. In these studies 16,000 natural and commercial chemical compounds have been screened for their anticancer

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activity and only one compound, salinomycin (**SAL**) (Scheme 1), an antibiotic belonging to a large group of natural polyethers isolated from *Streptomyces albus*, has been found to selectively kill breast cancer stem cells (CSCs) with 100-fold greater activity than the known anticancer agent – paclitaxel [19]. Extensive research work has been undertaken to explain the unusual anticancer properties of this compound, because CSCs play crucial role in tumour progression, chemoresistance and recurrence [20]. It has been also shown that **SAL** was able to induce apoptosis by multiple mechanisms in many human cancer cells resistant to cell death, but not in normal cells [21]. **SAL** showed strong inhibition of proliferation, migration and invasion of cancer cells, such as osteosarcoma, hepatocellular carcinoma, cholangiocarcinoma and gastric cancer [22–25]. Until now the synthesis and biological evaluation of various amides, esters and *O*-acylated derivatives of **SAL** have been described. Results of the tests have clearly shown that the obtained **SAL** derivatives exhibited potent anticancer activity against different human cancer cell lines, including drug-resistant cell lines [26–31].

Since 2012, **SAL** has been approved for testing in clinical studies on patients with invasive head, neck, breasts and ovary carcinoma. The results have shown inhibition in progress of the disease over an extended period of time. Acute side effects were rare and the serious long-term adverse side effects were not observed [32].

Recently, it has been proved that combination therapy with **SAL** and **FUra** had a synergistic anticancer effect against liver cancer both *in vitro* and *in vivo* [33]. This interesting observation inspired us to obtain and evaluate biological conjugates of these compounds, in which these compounds will be connected by covalent bond. Based on the idea that cancer cells are killed by **FdU** and **SAL**, we synthesized two different conjugates of **FdU** and **SAL** to enhance the anticancer activity of starting compounds.

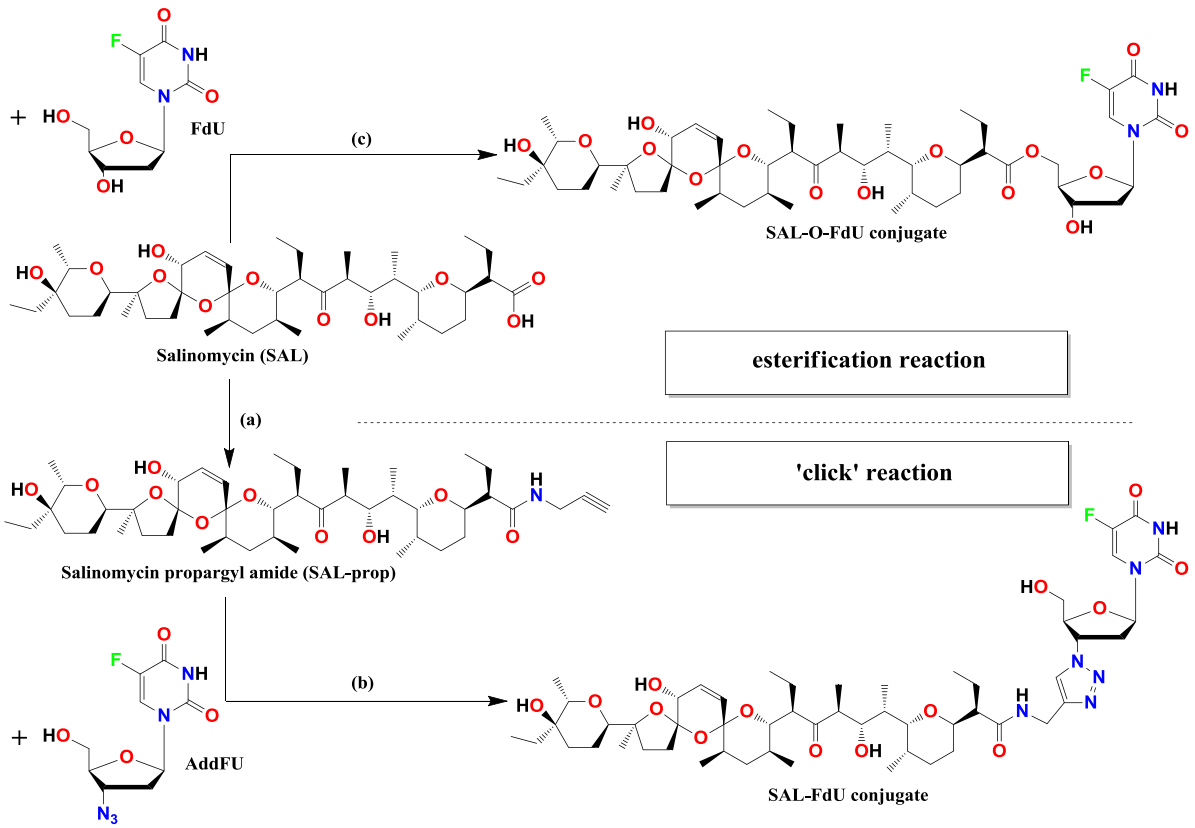
The present paper describes two efficient methods for the synthesis of two new bioconjugates. The first method is based on the 'click' chemistry and involves the copper(I) catalysed 1,3-dipolar Huisgen cycloaddition reaction. The 'Click' chemistry has been previously often applied for the synthesis of new nucleoside bioconjugates and it is one of the powerful tools for the generation of new drug candidates [1]. Many researchers used the 'click' chemistry as a synthetic tool for generation of pharmalogically valuable drugs. In the 'click' reaction, salinomycin propargyl amide (**SAL-prop**) and 2′,3′-dideoxy-3′-azido-5-fluorouridine (**AddFU)** react directly giving the first conjugate (**SAL-FdU**). In the second method two fragments **FdU** and **SAL** are joined by the ester bond in mild reaction conditions, to give **SAL-O-FdU** conjugate.

The structures of the compounds obtained were characterized using elemental analysis, FT-IR and NMR and ESI MS methods. The *in vitro* anticancer activity of these compounds against seven drug-sensitive and drug-resistant human cancer cell lines, as well as their antibacterial activity, especially against MRSA and MRSE hospital strains, were determined and discussed.

## 2. Results and discussion

### 2.1. Chemistry

The purity and structures of obtained compounds were determined on the basis of elemental, FT-IR, NMR and ESI MS analysis. The $^{1}H$ and $^{13}C$ NMR signals were assigned using one- and two-dimensional ($^{1}H–^{1}H$ COSY, $^{1}H–^{13}C$ HETCOR, $^{1}H–^{13}C$ HMBC) spectra. $^{1}H$ and $^{13}C$ as well as 2D NMR spectra of both conjugates are included in the Supplementary material (Figs. S1–S5 and




**Scheme 1.** *Reagents and conditions*: (a) DCC, HOBt, propargylamine, $CH_2Cl_2$/THF (3/1), 0 °C, 1 h; then rt,24 h; (b) $CuSO_4$, sodium ascorbate, dioxane/water (3:1); rt, 12 h; (c) DCC, PPy, *p*-TSA, $CH_2Cl_2$, 0 °C,6 h; then rt, 18 h. Time for completion of the reaction is indicated by TLC.