Bioorganic & Medicinal Chemistry Letters 23 (2013) 5053–5056

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

One-pot synthesis and cytotoxicity studies of new Mannich base derivatives of polyether antibiotic—Lasalocid acid

Adam Huczyński [a,*], Jacek Rutkowski [a], Izabela Borowicz [a], Joanna Wietrzyk [b], Ewa Maj [b], Bogumil Brzezinski [a]

[a] Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznan, Poland
[b] Ludwik Hierszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 123, 53-114 Wrocław, Poland

ARTICLE INFO

Article history:
Received 27 June 2013
Revised 16 July 2013
Accepted 17 July 2013
Available online 26 July 2013

Keywords:
Mannich bases synthesis
Lasalocid acid derivatives
Structural and spectroscopic studies
Antiproliferative activity
Polyether ionophores
Anticancer properties

ABSTRACT

Seven Mannich base derivatives of polyether antibiotic Lasalocid acid (**2a**–**2g**) were synthesized and screened for their antiproliferative activity against various human cancer cell lines. A novel chemoselective one-pot synthesis of these Mannich bases was developed. Compounds **2a**–**2c** and **2g** with sterically smaller dialkylamine substituent, displayed potent antiproliferative activity (IC_{50}: 3.2–7.3 μM), and demonstrated higher than twofold selectivity for specific type of cancer. The nature of Mannich base substituent on C-2 atom at the aromatic ring may be critical in the search for selectivity towards a particular cancer cell.

Lasalocid acid (**1**) (Scheme 1) isolated from *Streptomyces lasaliensis* is a representative of naturally occurring polyether ionophore antibiotics. The interest in this class of compounds stems from the fact that they show a very broad spectrum of bioactivity, for example, antibacterial, antifungal, antiparasitic and antiviral activity.[1–3] Recently it has been found that polyether ionophores might be also important chemotherapeutic agents for the treatment of cancer. These compounds have shown potent activity against the proliferation of various cancer cells, including those that display multidrug resistance (MDR), and cancer stem cells (CSC).[3,4] The discovery of strong anticancer properties of one of polyether antibiotics—salinomycin has received much attention in recent years.[4–11] The anticancer activity of polyether ionophores may be a consequence of the induction of apoptosis leading to cell death, arresting cell cycle progression, induction of the cell oxidative stress, loss of mitochondrial membrane potential, reversion of MDR, synergistic anticancer effect with other anticancer drugs, etc.[4,5] It is also worth noting that clinical studies of salinomycin on patients with metastatic breast cancer or metastatic head and neck cancers have been performed recently by Naujokat et al.[12] The patients with cancers treated with salinomycin showed partial tumor regression with only minoracute side effects. Thus, the preliminary results have permitted determination of a drug dosage that yields clinically significant benefits in the absence of excessive toxicity.[12] These interesting properties of polyether ionophores inspired our group to study the cytotoxic activity of **1**. Very recently we have found that **1** and its complexes with amines are strong cytotoxic agents towards cancer cell lines.[13] The cytostatic activity of these compounds was greater than that of cisplatin, indicating that lasalocid and its derivatives can be promising candidates for new anticancer drugs.[13]

Our previous investigation of semi-synthetic derivatives of different polyether ionophores has shown that the chemicalmodification of polyether antibiotics can change the ability and the selectivity of metal cations binding, modifying the mechanism of the cation transport and leading to new antibacterial and anticancer active compounds.[14–21]

As a continuation of these studies, in this contribution we present an effective one-pot method for the synthesis of *ortho*-phenol Mannich base derivatives of **1** (Scheme 1). To investigate the effect of different amine substitutions instead of the carboxylic group in the **1** molecule on bioactivity, we obtained seven new Mannich derivatives **2a**–**2g**. The structures of the new compounds **2a**–**2g** were evaluated using spectroscopic methods. In thepresent study, their antiproliferative activity has been tested in vitro using human breast adenocarcinoma (MCF-7), human lung adenocarcinoma (A549), human colon carcinoma (HT-29), murine leukaemia

* Corresponding author.
E-mail address: adhucz@amu.edu.pl (A. Huczyński).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.07.040

(P388), normal murine embryonic fibroblast (BALB/3T3) as well as human lung microvascular endothelial (HLMEC) cell lines.

Lasalocid sodium salt was isolated from premix-Avatec (commercial veterinary feed additive). Lasalocid acid (**1**) was obtained from lasalocid sodium salt by the extraction with H_2SO_4 (pH 1.5) in CH_2Cl_2 as described previously.[13]

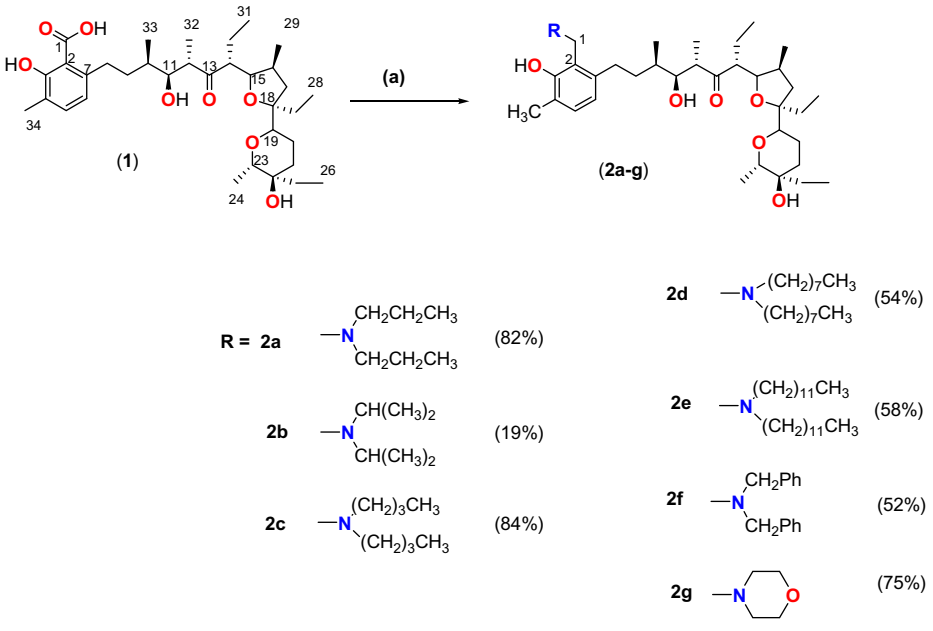
The synthesis of *o*-aminomethyl derivatives of phenols (Mannich bases) has been of interest in organic chemistry because it has led to new compounds of very important properties and many potential applications. Alot of them are used as complexing agents, catalysts or components of metal catalysts, intermediates in organic synthesis and biologically active compounds.[22–27]

Developing the synthesis of **1** Mannich base derivative is challenging because **1** is very sensitive to acidic and basic conditions and heating. Furthermore, molecule **1** includes many different functional groups (i.e., carboxylic, ketone, aromatic, etheric hydroxyl groups) and the synthetic procedure had to be highly chemoselective. Taking this into regard, it was deduced that a Mannich reaction would be ideal for conversion of carboxylic group of **1** to the dialkylaminomethyl group since the reaction can be carried out under neutral conditions and it is compatible with all of the other functional groups of **1**.In the molecule **1** there are three types of reactive sites for Mannich reaction, that is, alkyl keto group, positions 5 and 6 in aromatic ring and the carboxylic group. It has been shown that aminomethylation of phenols is achieved with formaldehyde and amines under acidic conditions using the Mannich reaction, which occurs readily in *ortho*- and *para*-positions affording substituted phenols. It has been also shown that alkyl keto group readily reacts under acidic conditions.[22–27] Because **1** is very unstable in acidic environment and higher temperatures, we chose mild conditions for the Mannich reaction using only three substrates (Lasalocid acid (**1**), paraformaldehyde and appropriate secondary amine 1:5:5) to perform one-pot reaction in toluene solution under reflux. This method was veryefficient and gave seven new Mannich derivatives (**2a**–**2g**) with good yields.[28] The lowest 19% yield was for the reaction between **1** and diisopropylamine because it has a highly sterically crowded molecule. The yields of the other reactions varied from 52% up to 84%. The respective synthesis pathways and yields are summarized in Scheme 1.

All Mannich bases (**2a**–**2g**) can be easily isolated in pure form after dry column vacuum chromatography on silica gel. The structures of all products (**2a**–**2g**) were determined using the ESI-MS, FT-IR, ^{1}H and ^{13}C NMR methods and are discussed below.[28] The ^{1}Hand ^{13}C NMR signals were assigned using two-dimensional spectra such as COSY, HETCOR, NOESY, HMBC, shown in the Supplementary data.

The products of the chemoselective one-pot reaction of **1** with formaldehyde and respective amine through the decarboxylation process are very well identified using the spectroscopic methods. In Figure S1 (Supplementary data) the FT-IR spectrum of **1** is compared with that of its exemplary Mannich base **2c**. In the spectrum of **2c**, the band assigned to the ν(C=O) stretching vibrations of the carboxylic group in the spectrum of **1** at 1652 cm^{-1}, vanishes completely, indicating the absence of carboxylic group with the formation of therespective Mannich base **2c**. The band assigned to the ν(C=O) vibrations of the ketone group in FT-IR spectra of **1** and **2c** is present at 1712 cm^{-1} indicating that Mannich reaction was chemoselective and no transformation of the ketone group occurs. In the ^{13}C NMR spectra of **2a**–**2g** Mannich bases, the most characteristic signal of C(1) atom of the methylene group at N atom is observed about 54.3 ppm. The presence of this signal and the absence of the signal of C(1) atom of the carboxyl group, observed in the spectrum of (**1**) at 173.2 ppm, proved decarboxylation of **1** during the synthesis. The connection of the salicylic aromatic ring moiety was carried out on the basis of two- and three-bond long-range correlation detected inthe HMBC spectrum. The correlation of the proton of methylene group C(1)H$_2$ singlet at ca 3.78 ppm with the ^{13}C NMR signals at ca. 119 ppm (C-2), 157 ppm (C-3) and 140 ppm (C-7) led to the conclusion that the aminomethyl group is present at C-2 atom (Scheme 1).

The reason why polyether antibiotics exhibit several pharmacological and biological effects is their ability to form lipid-soluble pseudo-cyclic complexes with metal cations and transport them through cell membranes disturbing their natural Na^+/K^+ ion balance.[1–4] Thus, before discussing biological activity of **1** derivatives it is important to study their ionophoretic properties. Therefore one of the obtained Mannich bases, **2g** has been used by us for the complexation of monovalent cations such as Li^+, Na^+ and K^+.



Scheme 1. Reaction of lasalocid acid: (**1**) with (**a**): paraformaldehyde and appropriate secondary amine (1:5:5 molar ratio) in toluene under reflux (5 h). Yields of the products are also given.