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Research paper

Evaluation of toxicity on epithelial and tumor cells of biaryl dipeptide tyrosines

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ABSTRACT

We report a method to obtain biaryl dipeptide tyrosine via Suzuki–Miyaura and alkynyl dipeptide tyrosine by Sonogashira cross-coupling reactions. Analysis of the biological action of biaryl dipeptide tyrosine 4d compound showed its ability to impair the metabolism and proliferation of SK-Mel-28 human melanoma lineage cells, independently of mitochondrial membrane depolarization, apoptosis and necrosis. Moreover, 4d compound did not cause toxicity to human umbilical vein endothelial cells (HUVEC), suggesting its toxic specificity to cancer cells.

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1. Introduction

Structural component of proteins and peptides, amino acids have been used as building blocks in the synthesis of more complex molecules and biologically active compounds, this fact is due to the low cost of amino acids, high availability and low toxicity. In this sense the tyrosine, a nonessential amino acid, but proteinogenic, is one of the few aromatic amino acids and being the only carrier of a phenolic nucleus, *ortho* director for alkylation and acylation reactions it is also used in obtaining of biaryl subunit via cross-coupling reactions [1].

The functionalization of tyrosine cores is a synthetic strategy that will achieve unnatural peptides, assigning a higher biological potential of this class of compounds, as an example of peptides containing tyrosine residues with known biological activity, the ustiloxin D, a potent antimitotic agent, isolated from the fungus *Ustaliginoidea virens* and recently synthetically exploited by Hutton and co-workers [2], as well as valorphin, with antiproliferative properties against tumor cells [3] (Fig. 1).

Moreover, amino acid derivatives have been employed as tracers in Positron Emission Tomography (PET) to detect neoplasms [4,5]. In this context, tyrosine derivatives have been considered promising tracers candidates because they take into account the lengthened half-live of tyrosine in comparison to other amino acids, such as methionine [4,6,7]. Indeed, the efficacy of fluoralkyltyrosine compounds as tracers has been shown in experimental models and human cancer [8–13].

Melanoma is a malignancy of melanocytes which are pigment-producing cells found in the skin, iris and rectum. The rate of malignant melanoma has increased in the last few years and it is estimated that there will be more than 73,000 new cases in 2015 in the United States (National Cancer Institute of NIH, 2015). Melanoma has a high metastatic index and patients with stage IV melanoma have a poor prognosis, with a mean survival of 8–10 months [14,15]. Advanced stages of disease are resistant to established therapeutic approaches, including chemotherapy, surgical excision, radiotherapy and immunotherapy [15].

In this sense, there is great demand for methods that allow the synthesis of a variety of functionalized tyrosine dipeptide derivatives. Herein, we report an efficient and general access method for the synthesis of biaryl dipeptide tyrosine derivatives via the Suzuki–Miyaura reaction and 3-alkynyl dipeptide tyrosine via Sonogashira coupling. Furthermore, we show that the **4d** derivative had an anti-proliferative effect on the human melanoma cell line

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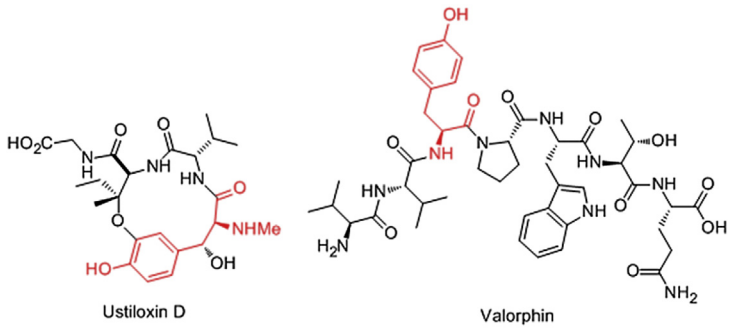

Fig. 1. Peptide tyrosine derivatives with known biological activity.

SK-Mel-28, with selectivity over normal human umbilical vein endothelial cells (HUVEC).

2. Results and discussion

2.1. Chemistry

Our strategy included the preparation of dipeptides **3a**–**c** through the formation of a peptide bond between tyrosine-methyl ester **1** and *N*-Boc-tyrosine **2**, using hydroxybenzotriazole (HOBt), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), triethylamine (TEA) and dichloromethane (DCM), in a 15 h reaction at room temperature. Using the same methodology it was possible to obtain the peptide **3a** in 57% yield with an iodine atom in the structure, as well as the peptides **3b** and **3c** containing one iodine atom in each aromatic ring, which were achieved in 48% and 57% yield respectively. From peptides core containing iodine we performed Suzuki–Miyaura or Sonogashira cross-coupling reactions [16,17].

In order to obtain an alkenyl dipeptide derivative, the peptide **3a** it was reacted with potassium trans-styryltrifluoroborate as substrate, using $Pd(OAc)_2$ in 10 mol%, K_2CO_3 as base in MeOH, the dipeptide **4a** was achieved in 89% yield. To obtain two different biarylic fragments, we prepared a Suzuki–Miyaura coupling reaction between the dipeptide **3b** and the potassium phenyltrifluoroborate salt, the reaction led the exclusive formation of desired cross-coupling product **4b** that after purification was isolated in 52% yield. Since iodine was still present in the peptide fragment, this allowed us to use a new cross-coupling reaction to get different biarylic subunits in peptides fragments. Therefore, dipeptide **4b** was reacted with potassium 3-thiophenetrifluoroborate salt under Suzuki–Miyaura conditions to give the compound **4c** in 42% yield.

The reactivity of the dipeptide **3b** was observed in the bis coupling reaction, when we used two equivalent of potassium 4-methoxyphenyltrifluoroborate salt and one equivalent of **3b** under appropriate conditions for Suzuki–Miyaura cross-coupling, just the compound **4d** was observed, after flash chromatography, the product could be obtained in 61% of yield.

The Suzuki–Miyaura products were obtained in the peptide fragments without phenol group protection; but when the Sonogashira cross-coupling reaction was performed, it was necessary to protect the phenol; the reaction without prior protection has led to the desired product in low yields. In this sense, using Pd(dppf) Cl_2CH_2Cl2 as a catalyst, CuI as a co-catalyst, TEA and THF, dipeptide **3c** and ethynyltrimethylsilane were reacted via a Sonogashira reaction to give the desired product **4e** in 89% yield in a single step (Scheme 1).

Peptide bond conditions: **2** (0.5 mmol) HOBt (1.1 eq), EDC (1.2 eq) **1** (0.5 mmol) TEA (1.2 eq), DCM, 15 h. Yield related to the isolated products.

2.2. Biological activity

A MTT assay showed that a solution of **4d** impaired the metabolism of SK-Mel-28 cells but not HUVEC. This effect was observed 24 or 48 h after incubation of the cells with two concentrations of the **4d** solutions (Fig. 2). As results from MTT assays may indicate alterations of mitochondrial membrane potential, we further evaluated if **4d** solutions could affect mitochondrial membrane depolarization. The results showed equivalent depolarization in HUVEC or SK-Mel-28 cells treated with PBS, vehicle or **4d** solutions (Fig. 3). Nevertheless, the positive control valinomycin, caused depolarization in both HUVEC and SK-Mel-28 cells, indicating the efficacy of the experimental procedure (Fig. 3).

A reduction in SK-Mel-28 cell metabolism may lead to impaired cell proliferation. Therefore cell proliferation assays were performed to further examine the biological action of solution **4d**. As shown in Fig. 4, incubation of SK-Mel-28 cells with both concentrations of **4d** led to lower cell numbers than when cells were incubated with vehicle, and an equivalent number of DMSO-incubated cells (positive control) (Fig. 4). Moreover, as expected, the proliferation of **4d**-treated HUVEC was similar to that of vehicle-treated cells and higher than that of DMSO-treated cells (Fig. 4), indicating that **4d** did not affect HUVEC proliferation.

Apoptosis or necrosis impair cell metabolism; therefore, we investigated if **4d** could induce apoptosis or necrosis in SK-Mel-28 cells. Results showed that **4d** did not induce cell death in the concentrations evaluated, as the percentage of SK-Mel-28 or HUVEC in apoptosis, late apoptosis or necrosis was similar in vehicle-treated or **4d**-treated cells (Fig. 5).

Together, these results indicate that the biaryl dipeptide tyrosine derivative is toxic to melanoma but not to epithelial cells. SK-Mel-28 lineage was chosen to test the toxicity because it is a malignant melanoma cell line. Melanoma development is aggressive, especially in the metastatic form, and no efficient treatments have been proposed until now [18]. Therefore, novel or complementary pharmacological approaches are needed for the treatment of melanoma. The results from this study show that further biological tests should be conducted to determine if biaryl dipeptide tyrosine derivatives could be used as therapeutic agents. It is important to mention that **4d** selectivity to SK-Mel-28 cells over HUVEC strengthens our data, as the endothelium consists epithelial cells covering the luminal surface of all vessels, and therefore, it is the first barrier to chemical agents during tissue distribution from the blood circulation [19,20].

The mechanism involved in the selectivity of **4d** has not been identified, but it is possible that cancer cells may uptake the

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