## Design, synthesis and pharmacological tests of leukotrienes a4 hydrolase inhibito...

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## Design, synthesis and pharmacological tests of leukotrienes A4 hydrolase inhibitors as potential targets of interest in cancer treatment

The paper " Design, Synthesis and Pharmacological Tests of Leukotrienes A4 Hydrolase Inhibitors" is a worthy example of a lab report on medical science. (E)-Resveratrol is a naturally occurring, potent LTA4 hydrolase inhibitor of high medical importance. Being based on stilbene skeleton, resveratrol can exist as both cis-(Z) and trans-(E) isomers, being the trans isomer the most commonly found in nature (Romero-Perez et al, 1996). Unfortunately, due to its low quantities in natural sources, it cannot be obtained in large amounts by extraction from plants, such as Vitis vinifera. Therefore, many synthetic pathways were developed to synthesize this highly promising compound. (Farina, Guiso, and Marra, 2002). One of the most efficient ways to achieve (E)-resveratrol is by employing the Heck reaction. The process is highly advantageous as it produces only the desired product in exceptional 99% yield:

Scheme 1: Synthesis of (E)-resveratrol using Heck reaction

As it is seen on scheme 1, the starting 3, 5-diacetoxy-stirene (1) was coupled with the para-iododerivative (2). Deacetilation of the formed stilbene derivative afforded the desired isomer of resveratrol.

The starting material (1) was prepared through the Wittig reaction followed by subsequent acetylation of the produced phenol as can be illustrated in the following diagram.

Scheme 2: Preparation of 3, 5-diacetoxy-stirene (1)

## While addressing the topic of trans-resveratrol, the research does not make

any contribution to the area of cis-resveratrol synthesis and application. (Farina, Guiso, and Marra, 2002).

To fill that gap, it is worth mentioning other methods of resveratrol synthesis. For example, Roberti, et al. (2003) synthesized a series of cis- and trans-resveratrol (Scheme 3) derivatives using the following Wittig reaction. Scheme 3: Synthesis of stilbene derivatives

The groups marked as " R" are both silicon and carbon-containing ethers. The silicon-containing groups were further removed and the ability to induce apoptosis in cells of HL60 promyelocytic leukemia as well as cell growth inhibition properties of produced cis and trans-resveratrol derivatives were investigated. In most cases, trans isomers were less effective than their cis counterparts. It was also established that the following two products stand out for showing the best results in the mentioned tests.

Scheme 4: Highly promising cis-Resveratrol derivatives

Comparing the accumulated results the authors concluded that structural changes in the stilbene backbone will undeniably lead to the production of powerful apoptosis-reducing agents.

The described research takes the concept of resveratrol based drugs to a new level, but do not mention the poor stability of cis-resveratrol (4). The information about cis-resveratrol is not as abundant as it is for trans isomer. Cis-resveratrol has shown antioxidant properties, antiplatelet properties and affect cytoplasmic calcium levels in vascular smooth muscle cells Cisresveratrol (4) has also shown to inhibit kinase activity, a factor related to cancer (Jayatilake et al., 1993) and has a significant modulatory effect on the nuclear factor kappa B signaling pathway (Leiro et al, 2005) and,

consequently, an important antioxidant role that may partially explain the cardioprotective effects attributed to long-term moderate red wine consumption. That was the main reason for our design of a cis-resveratrol analog (5) by modulative pharmacomodulation. (Scheme 5).

Scheme 5: cis-resveratrol (4) and it's analog (5) produced by modulative pharmacomodulation.

The compound preserves cis-resveratrol's essential structural requirements and does not have the possibility of isomerization like it happens with natural cis-resveratrol (Trela and Waterhouse, 1996). For the synthesis of the resveratrol analog (5), we need to prepare the corresponding nitro derivative (6) from the respective aldehyde (7) using the methodology developed by Dauzone (1986) as described in scheme 6.

Scheme 6

In order to prepare compound 5, we need to perform the Suzuki-Miyaura condensation (Beaumard et al., 2009) as described in scheme 7.

Scheme 7

After treatment of the nitro derivative 9 with sodium azide in DMSO (Quiclet and Zard, 2005) and deprotection, we should be able to obtain the desired compound 5 (Scheme 8).

Scheme 8

MTT assay:

To asses cell growth inhibition properties MTT assay will be conducted. This colorimetric test is possible to apply in this particular case as LTA4H is an enzyme that reduces MTT. To do that 96-well plates will be populated with studied cancer cells(5000/well). To produce consistent results, six

measurements should be done, repeating each concentration three times. At 370C MTT (5 mg/ml) for 4h will be added to the cells grown in various experimental conditions. As a consequence, purple formizan will be accumulated by the reduction of MTT in the mitochondria of the living cells. Formisan is stabilized by DMSO. The amount of reduced MTT provides information about the number of viable cells and, therefore, the effectiveness of the drug and cell proliferation.

The obtained formazan in its crystal state will be solubilized with DMSO and the absorbance measured at 570 nm using a Dynatech MR 5000 spectrometer. As this type of spectrometer is designed for 96-well plates it is possible to do all the measurements from 1. 7 to 2. 2 seconds depending on its input parameters.