Design and synthesis of fluorescent GPER ligands as useful tools in molecular biology and drug discovery process

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Breast and ovarian tumors are among the major cause of death in Western Countries' women. Estrogens play a pivotal role in the development of these hormone-sensitive tumors. Recently, a seven-transmembrane G-protein-coupled receptor (GPCR), named GPER, has been identified as membrane estrogen receptor able to mediate rapid estrogen signalling in a variety of normal and cancer cell types.[1] Different studies showed that GPER promotes the up-regulation of the oncogene *c-fos* and stimulates proliferative effects induced by estrogens and antiestrogen in cancer cells such as breast, endometrial and thyroid.[2] GPER is a 7 helices transmembrane protein (7TM) and its cellular localization is still a matter of debate: although it has been found within the Endoplasmic Reticulum membrane, further studies demonstrated the presence of this receptor on the cellular membrane. GPER has been associated with the proliferative effects induced by 17β-estradiol (E2) and a selective ligand of GPER, G-1, (Figure 1A) through a functional cross-talk with ERα in ovarian cancer cells, playing an important role in tamoxifen- resistant breast cancer cells.[3]

Taken together, these data indicate that GPER plays a role in a complex transduction network which mediates the biological responses to estrogens. However, the mechanism of activation of signals, the structure-function relationships, the pharmacological spectrum regarding GPER still suggest questions to be solved. The possibility to develop small molecular probes, to facilitate elucidation of mechanistic pathways and enable specific manipulation of the activity of GPER, provides an extraordinary tool in the complex field of drug discovery. Biological information can offer a more detailed scenery if the classical and efficient method of investigation, based on the exposition of cells to a fluorescent dye, such as one of the commonly used Alexa family,[3] can be flanked to the use of luminescent ligands of the protein under study.

In this communication we describe the synthesis of a family of small GPER ligands with intrinsic fluorescent properties. This approach can be regarded as a useful tool for exploiting structural changes of a protein upon interaction with specific target molecules and developing new targeted imaging agents for the intracellular receptor. We started in designing small molecules (Figure 2) showing the structural characteristics of some already known GPER ligands, such as the agonist G1 and the antagonist G15 (Figure 1B), but with fluorescent elements incorporated in their skeleton, trying to avoid alteration in their properties as GPER agonists or, better, antagonists. For the fluorescence, we have been inspired by the dipyrromethene boron difluoride (BODIPY) dyes (Figure 1C) as strongly UV-visible absorbing small fluorophores that exhibit relatively sharp fluorescence with high quantum yield,[4] are reasonably stable under physiological conditions and have been widely investigated as fluorescent probes for biological studies.

In Figure 3 the two synthetic pathways a) and b), for the preparation of compounds 1, are shown. This communication will describe the details of these syntheses, the results gained and the work that has to be ended. The design of the fluorescent GPER ligands has been supported by virtual screening of their potentially effective molecular skeletons.

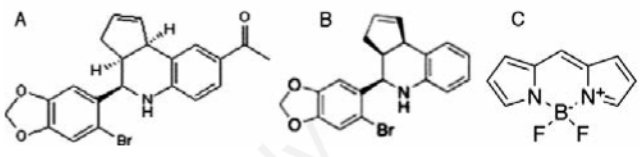

Figure 1. A: GPER agonist G-; B: GPER antagonist G-15; C: the skeleton of fluorescent BODIPY.

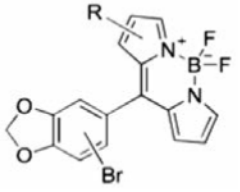

Figure 2. The general skeleton of the projected GPER fluorescent ligands.

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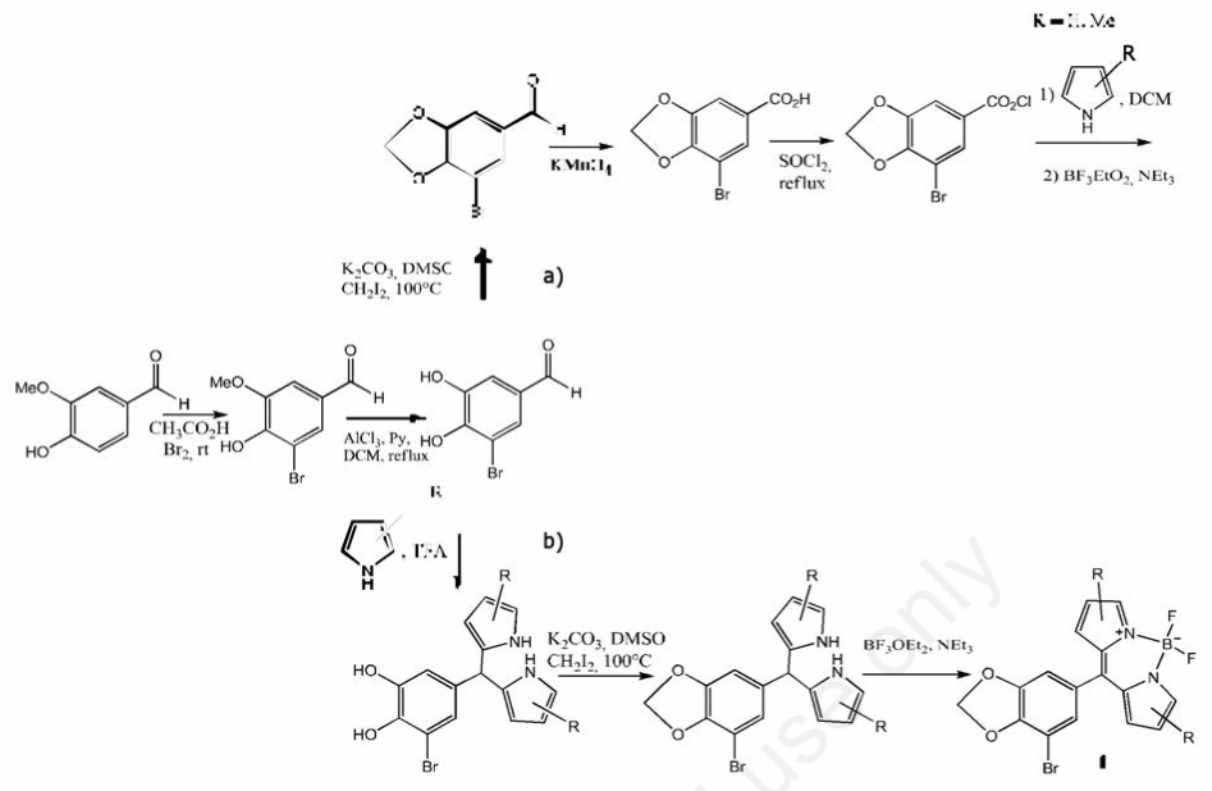

Figure 3. Synthetic pathways to fluorescent GPER ligands.

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