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Research paper

Spirotriazoline oxindoles: A novel chemical scaffold with *in vitro* anticancer properties

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ABSTRACT

The design and synthesis of a library of twenty-six spirotriazoline oxindoles and their *in vitro* evaluation as potential anticancer agents is reported. The antiproliferative activity of the synthesized compounds was assessed against four different cancer cell lines (HCT-116 *p53* $^{(+/+)}$, HCT-116 *p53* $^{(-/-)}$, MCF-7, and MDA-MB-231). Four spirotriazoline oxindoles showed selectivity against the four cancer cell lines tested over the non-cancer derived HEK 293T cell line. To characterize the molecular mechanisms involved in compound antitumoral activity, two spirotriazoline oxindoles were selected for further studies. Both compounds were able to induce apoptosis and cell cycle arrest at G0/G1 phase and upregulated p53 steady-state levels, while decreasing its main inhibitor MDM2, in HCT-116 cells. Importantly, cytotoxic effects induced by spirotriazoline oxindoles occurred in cancer cells without eliciting cell death in non-malignant CCD-18Co human colon fibroblasts. In addition, four spirotriazoline oxindoles showed selectivity against the triple-negative breast cancer cell line MDA-MB-231 with IC_{50} values of 3.5–6.7 μM. These results highlight the anticancer potential of spirotriazoline oxindoles, especially when dealing with aggressive and challenging triple-negative breast cancer.

1. Introduction

It is well established that cancer represents a major public health issue worldwide. In 2012, it was estimated 14.1 million new cancer cases and 8.2 million deaths [1], and the World Health Organization estimates a rise of 13.1 million cancer deaths until 2030. Specifically, breast cancer is the most common cancer in woman, and is the leading cause of cancer death in women aged 20–59 years in the USA [2]. In particular, a specific tumor subtype, known as triple-negative breast cancer (TNBC) is characterized as a more aggressive type of cancer with poorer prognostic. TNBCs are characterized by the lack of expression of estrogen and progesterone receptors, and absence of human epidermal growth factor receptor 2 overexpression, representing the hardest breast cancer to treat [3]. Although early detection and efficient therapies contributed to a decrease of cancer mortality in developed countries, overcoming drug intrinsic and acquired resistance represents a major challenge [4,5] and imposes a constant development of new molecules and the identification of novel targets, especially for TNBC patients [3].

Heterocycles possessing a spirooxindole framework are found in many natural products and medicinal agents with diverse biological activities [6–8]. The attractiveness of this type of scaffold in organic and medicinal chemistry is evident by the increasing number of publications on this topic over the last 10 years. This can be ascribed, at least partially, to the fact that the central spiro carbon atom imposes a conformational restriction to the structure that can be beneficial for ligand-target binding, and thus potentially promoting an increase in potency and/or specificity [7].

Furthermore, the reduced molecular flexibility displayed by the spirooxindole scaffold can also potentially lead to better pharmacokinetic properties [9]. Antiproliferative activity against cancer cell lines is one of the biological effects reported for spirooxindoles (Fig. 1) [10]. Specifically, CFI-400945 is a Polo-like kinase 4 (PLK4) inhibitor [11], compound (−)-**1** interferes with microtubule polymerization and arrests mitosis [12], and compounds MI-77301 [13] and SM13 [14] modulate p53 activity.

We have recently developed several small molecules showing potential anticancer activity [15–23], with a focus on novel five-membered ring spirooxindoles that act as p53 modulators (Fig. 2). Initial studies explored the spiroisoxazoline oxindole

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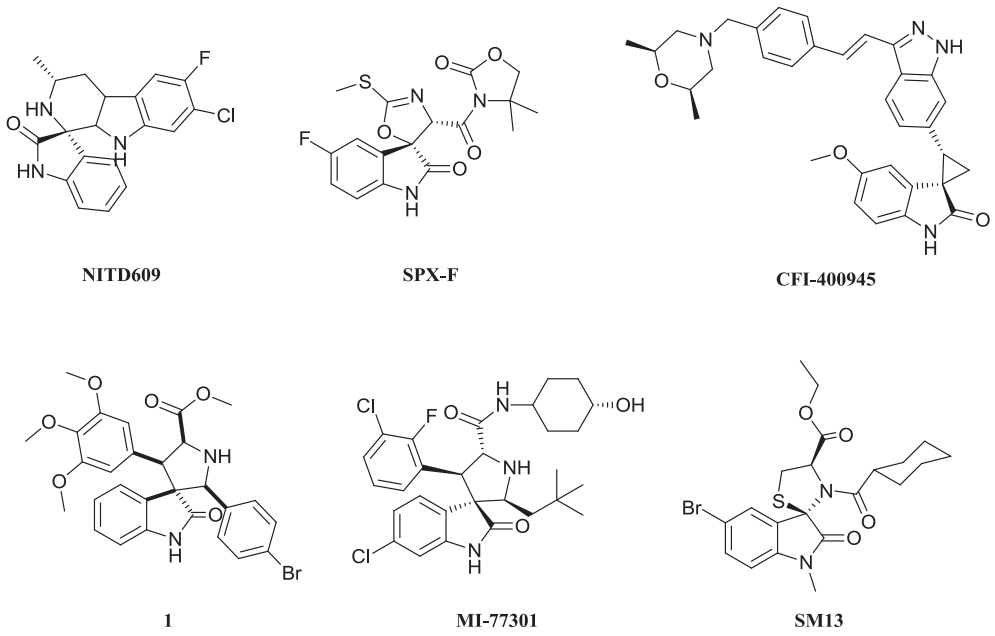

Fig. 1. Spirooxindoles with biological activity.

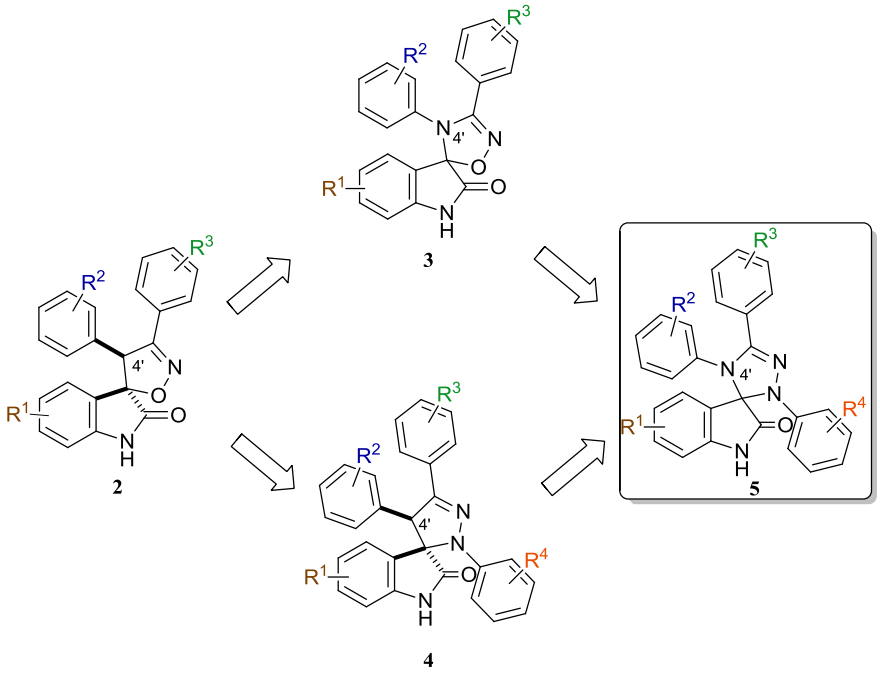

Fig. 2. Optimization strategy towards spirotriazoline oxindoles **5**.

scaffold **2** [21]. Then, the spirooxadiazoline oxindole scaffold **3** was obtained by replacing the chiral isoxazoline C4′ for a N4′ atom. Further optimization led to a spirooxadiazoline oxindole 15.6-fold more potent in HCT-116 colorectal cancer cells than the most active spiroisoxazoline oxindole previously developed by us [15]. We also explored the addition of a fourth lipophilic moiety to the main scaffold, by changing the isoxazoline oxygen by a *N*-phenyl group (spiropyrazoline oxindole scaffold **4**) [20,23]. These compounds were tested in two breast adenocarcinoma cell lines, MCF-7 and MDA-MB-231. One compound, spiropyrazoline oxindole **4a**, was found to be at least 13.7-fold more potent against MCF-7 cells over MDA-MB-231 cells (TNBC cell line), and the non-cancer derived human embryonic kidney HEK 293T cell line (Fig. 3).

Interestingly, spiropyrazoline oxindole **4b** showed approximately the same potency against both breast cancer cell lines, but maintained selectivity (>4.5-fold) over the HEK 293T cell line. This observation suggests that it would be possible to obtain differential selectivity in breast cancer cell lines depending on the position of the halogen in the oxindole moiety.

Since both strategies gave rise to improved and/or more selective compounds, we decided to apply and explore simultaneously both optimization strategies: replacing the chiral isoxazoline C4′