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Short communication

# Synthesis of novel spiropyrazoline oxindoles and evaluation of cytotoxicity in cancer cell lines

Ângelo Monteiro, Lídia M. Gonçalves, Maria M.M. Santos*

*Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal*

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ABSTRACT

A series of novel spiropyrazoline oxindole derivatives was synthesized by 1,3-dipolar cycloaddition reaction. The compounds were screened for their in vitro cytotoxic activity against MCF-7 breast cancer cell line (estrogen receptor positive (ER+) and human epidermal growth factor receptor 2 negative (HER2−)). Of the nineteen spiropyrazoline oxindoles tested, six compounds have a $GI_{50}$ below 12 μM The most potent compounds in this series were also evaluated against MDA-MB-231 breast cancer cell line (ER− and HER2−). Two spiropyrazoline oxindoles were highly selective between MCF-7 tumor cells and MDA-MB-231 tumor cells. More importantly, they were noncytotoxic against HEK 293T non tumor derived cell lines.



## 1. Introduction

Cancer is one of the leading causes of mortality worldwide, causing 7.6 million deaths in 2008. Moreover, World Health Organization projects a rise in deaths from cancer to 13.1 million in 2030 [1]. Despite a more sophisticated understanding of the etiology of cancer and a greater emphasis placed on early detection of the disease, overall mortality rates from cancer have not diminished greatly and are not expected to decrease. As such, cancer continues to pose a major threat to human health and further research regarding new therapeutic strategies that more effectively combat cancer are needed. Furthermore, the increase cases of multidrug resistance (MDR) make a challenge for the development of new drugs a milestone on the treatment of various types of cancers (e.g. blood, breast, ovarian, lung, and lower gastrointestinal tract cancers). Various MDR mechanisms observed in cancer cells as well as various strategies developed to overcome these mechanisms have been extensively studied during the last few decades to enhance the efficacy of chemotherapy by suppressing or evading the MDR mechanisms [2].

We previously reported the potential use of spiroisoxazoline oxindoles as anticancer agents [3]. In fact, the spirooxindole system is the core structure of a variety of medicinal agents and natural products [4,5]. Spirooxindole derivatives were described with different biological activities, ranging from MDM2 antagonists, ion channel blockers and anti-inflammatory agents to antimalarials (Fig. 1) [6–9]. As a consequence, there is a huge interest in industry and academia to develop novel spirooxindoles with interesting biological activities. Pyrazoline is also a privileged unit in medicinal chemistry. We now report the study of spiropyrazoline oxindoles, containing a five membered ring (pyrazoline) with one more aromatic substituent ($R^4$) (oxygen atom in isoxazoline ring was replaced by a *N*–Ar group). To perform a structure-activity study, we synthesized and evaluated the antiproliferative activity of a small library of novel spiropyrazoline oxindoles containing different substituents at the pyrazoline ring and in the aromatic ring of the oxindole moiety (Fig. 2). Two breast cancer cell lines, one non-invasive estrogen receptor (ER) positive (MCF-7) and, one invasive ER-negative (MDA-MB-231), both derived from a metastatic adenocarcinoma on the mammary gland, were chosen as models for testing the new synthetized molecules. The ER-negative breast cancer [10] is specifically studied due to a lack of cellular targets compared with ER-positive breast cancer MCF-7 cell line, which can be effectively inhibited by targeting the estrogen receptor with anti-estrogen agents. The ER-positive breast cancer cells are described to often develop MDR [2,11]. This makes very important the search for potential new anticancer drugs independent from estrogens receptors to treat cancer.

* Corresponding author. Medicinal Chemistry Group, iMed.ULisboa, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.
*E-mail address:* mariasantos@ff.ul.pt (M.M.M. Santos).

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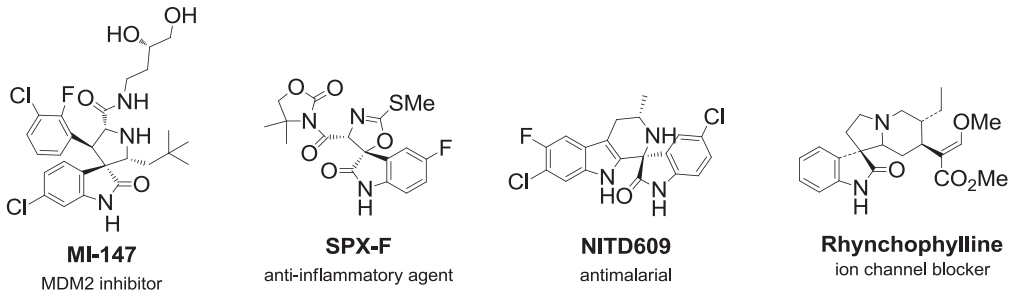



**Fig. 1.** Selected spirooxindoles with biological activity.

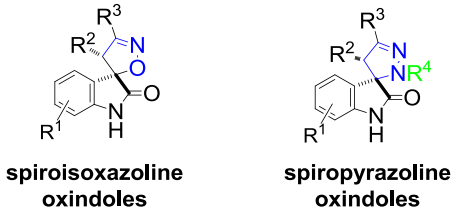



**Fig. 2.** Chemical structure of spiroisoxazoline and spiropyrazoline oxindoles.

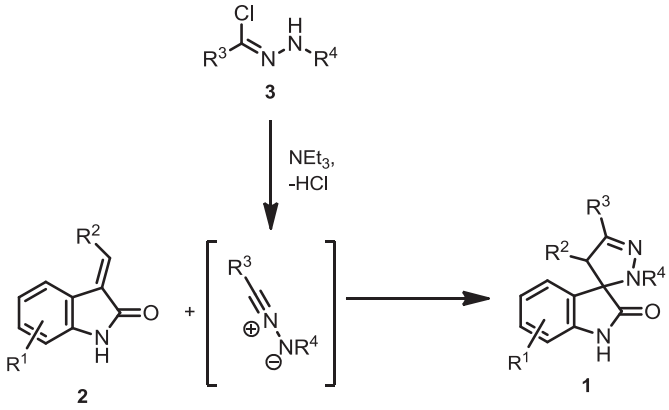
## 2. Results and discussion

### 2.1. Chemistry

Spiropyrazoline oxindoles **1** were synthesized by 1,3-dipolar cycloaddition reaction, one of the most versatile methods to obtain 5-membered heterocycles, between nitrile imines and 3-methylene indolinones **2** (Scheme 1). The nitrile amines were prepared in situ from hydrazonyl chlorides **3**.

The 3-methylene indolinones **2** containing aromatic groups at $R^2$ were synthesized by aldolic condensation of substituted indolin-2-ones with different aromatic aldehydes in the presence of piperidine in yields between 92 and 98% [12].

Specifically, the 3-methylene indolinone required for the synthesis of compounds **1a**–**c** and **1e** was obtained by aldol condensation between 5-chloro isatin with acetophenone in basic medium, followed by dehydration using dilute alcoholic hydrochloric acid as described in the literature [13] and, the 3-methylene indolinone required for the synthesis of compounds **1d** was easily prepared by Wittig reaction of 5-chloro isatin with (carbethoxymethylene)triphenylphosphorane in 92% yield [14].




**Scheme 1.** Strategy to obtain spiropyrazoline oxindoles **1**.

The hydrazonyl chlorides **3** required for the dipolar cycloaddition were obtained from reaction of *N*-chlorosuccinimide-dimethyl sulphide complex with the appropriate *N*-arylhydrazones at −78 °C [15].

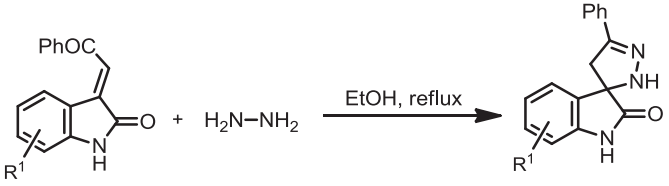
We started by synthesizing spiropyrazoline oxindoles **1a**–**c**, with the nitrogen of the pyrazoline ring unprotected, by nucleophilic addition of hydrazine to the appropriate 3-methylene indolinones **2** (Scheme 2) [13].

Then, spiropyrazoline oxindoles **1d**–**s** were synthesized from the appropriate 3-methylene indolinones **2** with hydrazonyl chlorides **3** in the presence of triethylamine (Scheme 3). The active nitrile amine required for the 1,3-dipolar cycloaddition was generated in situ by dehydrohalogenation of the corresponding hydrazonyl chloride in the presence of base. The reaction was regioselective, with the carbon end of the dipole adding to the β-position of 3-methylene indolinones. The relative configuration of the final products was established by comparison of their NMR spectra with NMR spectra of other spiropyrazoline oxindoles described in the literature, with published X-ray crystallography structure [16]. Using compound **1n** as an example, the proton chemical shift observed for the hydrogen of the pyrazoline ring was 5.14 ppm and the carbon chemical shift observed for the spiro carbon was 77.90 ppm.

### 2.2. In vitro cytotoxicity

The in vitro cytotoxicity of compounds **1a**–**s** was evaluated by MTT assay in MCF-7 (human breast adenocarcinoma) tumor cell line. The $GI_{50}$ values obtained (Table 1) allows the following observations:

1. Compounds **1a**–**c**, with an unsubstituted nitrogen and hydrogen at $R^2$, are inactive against MCF-7 cell line at tested concentrations ($GI_{50} > 100$ μM).
2. Of the nineteen compounds tested, six compounds (**1j**–**1o** and **1q**–**1r**) have a $GI_{50}$ below 12 μM. These results show that the substitution of an isoxazoline ring [3] by a pyrazoline ring leads to an increase of activity as anticancer agents.
3. The order of inhibitory activity against MCF-7 cell line depends in significant extent on the nature of the substituent at $R^2$. Compounds **1d** and **1e** with a $CO_2Et$ and a COPh substituent,




**Scheme 2.** Synthesis of spiropyrazoline oxindoles **1a**–**c**.