New *N*-substituted spiropyrazoline oxindoles as anticancer agents: Synthesis and stability evaluation

Ricardo Ferreira [(1)], Ana Paula Francisco [(1)], Maria M. M. Santos [(1)]

(1) Medicinal Organic Chemistry Group, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal

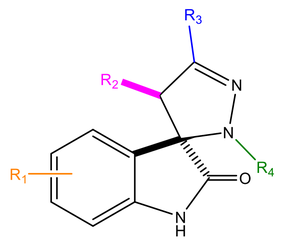
E-mail: ricardojferreira@edu.ulisboa.pt ; Group webpage : https://medorgchemlab.wixsite.com/medorgchemgroup

Introduction

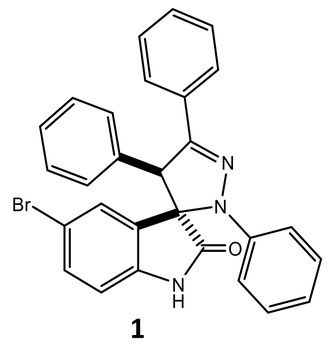
Spirocyclic oxindoles are found in many synthetic and natural products with interesting biological activities. In the last years, our research group has been involved in the development of novel spiropyrazolines oxindoles with anti-proliferative activity against cancer cell lines, while not being toxic against non-cancer cells. One compound (**1**) emerged with moderate antiproliferative activity against breast, ovarian and colon cancer cell lines (MCF-7, A2780 and HCT-116) [1,2]. Moreover, this compounds interacts with BSA with a Kb value of 3.14 x 10^6 M^{-1} indicating that it can be efficiently transported by serum proteins in blood [3].

Main objectives

- Explore the effect on activity of adding substituents at the indole nitrogen of compound **1**, a position still unexplored in this scaffold;
- Improve the physico-chemical properties of compound **1**, which is hydrofobic, by introducing hydrogen bond donor and acceptor chains.



Positions already exploited



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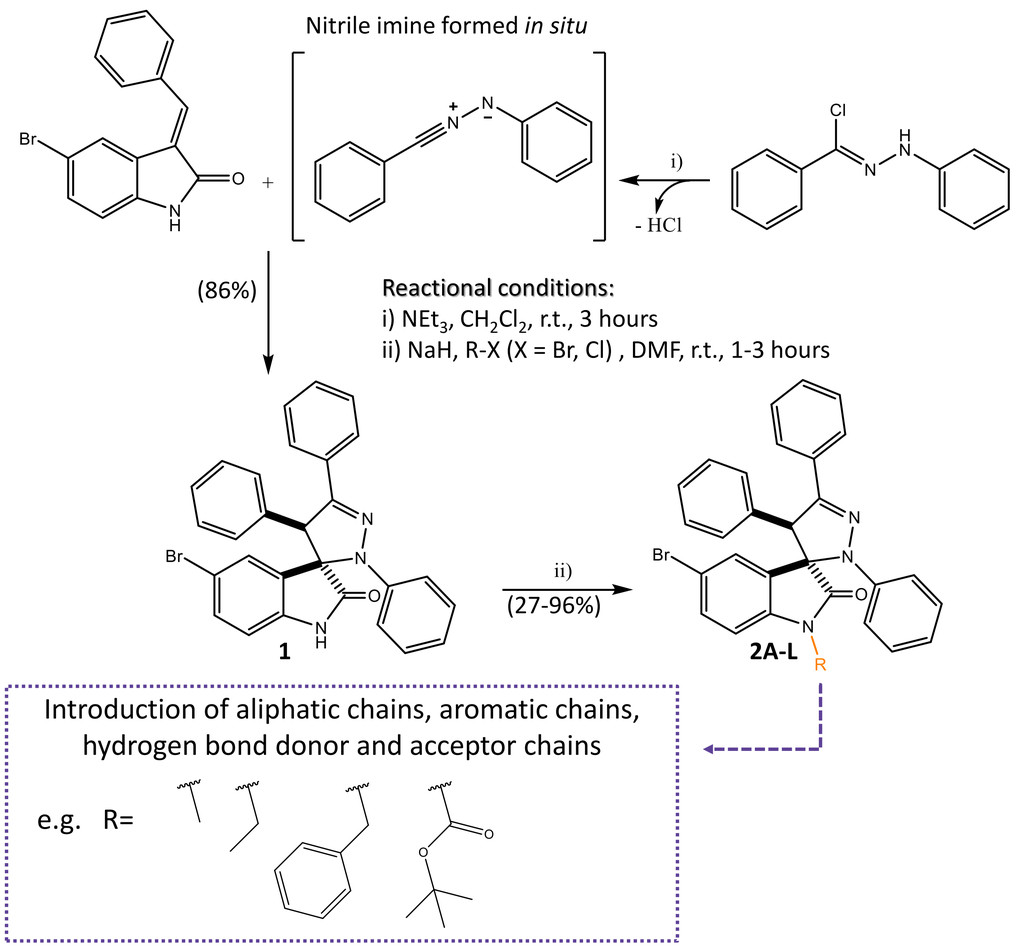
- ✓ Active against breast cancer cell line (MCF-7), ovarian cancer cell line (A2780) and colon cancer cell line (HCT-116)
- ✓ Induces apoptose and cell cycle arrest at G0/G1 phase
- ✓ Induces autophagy in ovarian cancer cells (A2780)
- ✓ Upregulates p53 steady-state levels
- ✓ Low toxicity in normal cells

Synthesis of novel compounds



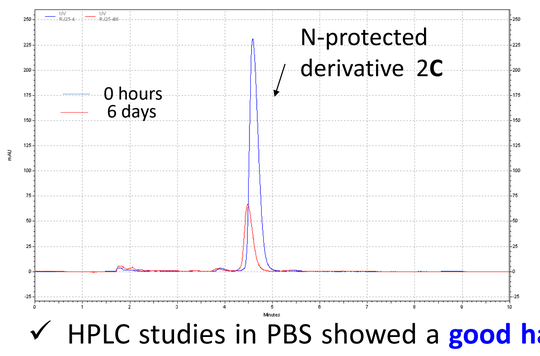
✓ Library of **12** novel spiropyrazoline oxindoles were synthetized

The N-protected derivative with a hydrogen bond donor chain (**2C**) was selected for stability studies

Stability studies

- **Stability in phosphate-buffer saline (PBS), pH=7.4**

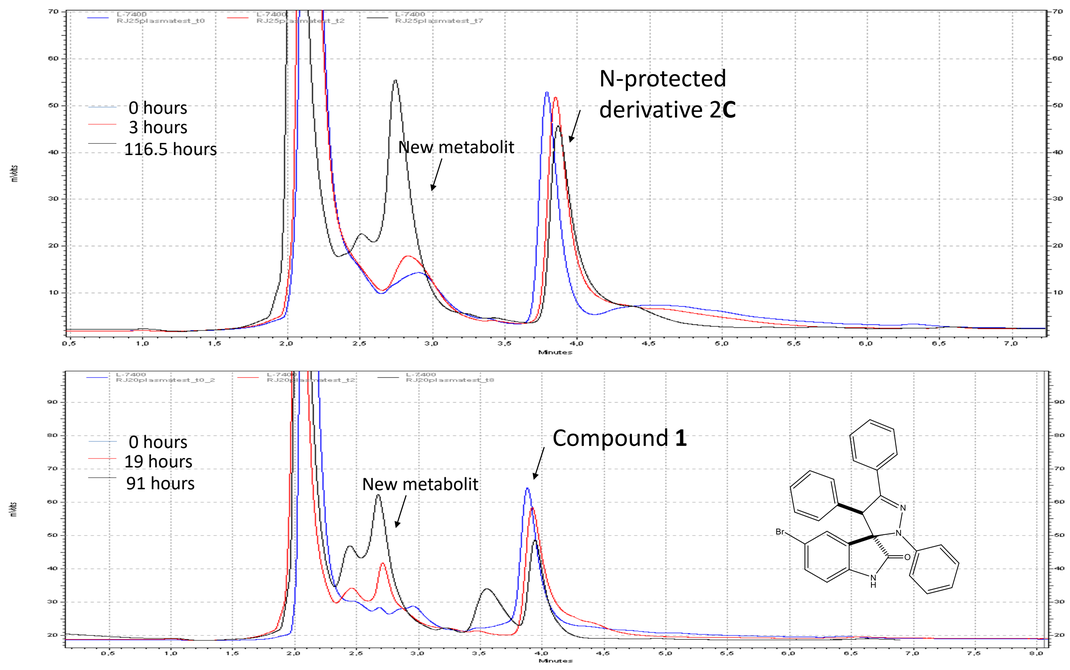
Assay conditions: 30µL f a 10^{-2}M stock solution + 10mL of PBS (0.01M, pH = 7.4, 20%ACN), 37°C, λ=240nm.



✓ HPLC studies in PBS showed a **good half-life** for compound **1** (on the right) and the N-protected derivative **2C** (on the left), confirming **good stability under these conditions.** After 6 days a 71% degradation was observed for compound **2C** and 62% degradation for compound **1**.

- **Stability in human plasma**

Assay conditions: 2mL of human plasma + 0.5mL of PBS + 5µL f a 10^{-2}M stock solution, 37°C. 200µL of reaction mixture+ 400 µL of ACN, centrifuged at 13.000 rpm, 10min, λ=240nm.



✓ In plasma compound **2C** ($t_{1/2}$ about 145 hours) has a **similar half-life as in PBS**, indicating that its degradation in plasma is only due to pH and not promoted by plasma constituents as proteins and enzymes; while compound **1** ($t_{1/2}$ about 71.7 hours) degrades much faster**.**

Final considerations

- ✓ A new library of spiropyrazolines was prepared
- ✓ Stability studies showed that the hit compound **1** and the N-protected derivative **2 are very stable** in PBS (pH=7.4); while compound **2C** is more stable than the hit compound (3-fold faster degradation in plasma than in PBS) in human plasma
- ✓ The synthesized compounds are currently being evaluated against several cancer cell lines (MCF-7, HCT-116 and A2780) to obtain novel anti-cancer agents

Acknowledgements

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References

[1] Monteiro Â, et al. Eur. J. Med. Chem. **2014**, 79, 266. [2] Nunes R.C., et al. Eur. J. Med. Chem. **2017**, 139, 168. [3] Raposo L.R, et al. Bioor. Med. Chem. **2021**, 30, 115880.