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

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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 spirooxindoles 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 hydrophobic, by introducing hydrogen bond donor and acceptor chains.

Synthesis of novel compounds

![Synthesis scheme](image)

![Synthesis scheme](image)

![Synthesis scheme](image)

Introduce of aliphatic chains, aromatic chains, hydrogen bond donor and acceptor chains e.g. R=

- Library of 12 novel spirooxindoles 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 + 10^-7M stock solution + 10μL of PBS (0.01M, pH = 7.4, 20%ACN), 37°C, λ=240nm.
  - Active against breast cancer cell line (MCF-7), ovarian cancer cell line (A2780) and colon cancer cell line (HCT-116)
  - Induces apoptosis 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

- 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^-7M stock solution, 37°C. 200μL of reaction mixture+ 400 μL of ACN, centrifuged at 13.000 rpm, 10min, λ=240nm.

  - In plasma compound 2C (t1/2, 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 (t1/2, about 71.7 hours) degrades much faster.

Final considerations

- A new library of spirooxindoles 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

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References


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