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Research paper

Discovery of pyrrolospirooxindole derivatives as novel cyclin dependent kinase 4 (CDK4) inhibitors by catalyst-free, green approach

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ABSTRACT

Aiming to develop a new target for the anticancer treatment, a series of 5′H-spiro[indoline-3,4′-pyrrolo[1,2-a]quinoxalin]-2-ones has been synthesized by simple, highly efficient and environmentally friendly method in excellent yields under catalyst-free conditions using ethanol as a green solvent. A simple filtration of the reaction mixture and subsequent drying affords analytically pure products. The synthesized derivatives were evaluated for their antiproliferative activity against five different human cancer cell lines, among the congeners compound **3n** showed significant cytotoxicity against the human prostate cancer (DU-145). Flow cytometric analysis revealed that this compound induces cell cycle arrest in the G0/G1 phase and Western blot analysis suggested that reduction in Cdk4 expression level leads to apoptotic cell death. This was further confirmed by mitochondrial membrane potential ((ΔΨm), Annexin V-FITC assay and docking experiments. Furthermore, it was observed that there is an increase in expression levels of cyclin dependent kinase inhibitors like Cip1/p21 and Kip1/p27.

1. Introduction

Spirocyclic compounds are notable for their molecular architecture as well as the prominent biological activities [1]. Especially, spirocyclic oxindoles are attractive synthetic targets because of their widespread occurrence in various natural products and biologically active molecules [2,3]. The structural rigidity imparted by the spiro-carbon causes conformational restrictions and this may influence the binding of such compounds to the biological targets favorably. A selected assortment of natural products incorporating the spirooxindole unit is depicted in Fig. 1. Spirotryprostatin A and B are two natural alkaloids isolated from the fermentation broth of *Aspergillus fumigates*, that have been identified as promising inhibitors of microtubule assembly [4]. Most of these spirooxindole alkaloids were isolated from the Apocynaceae and Rubiacae species, which play important roles in medicine [5], some other important spirooxindole natural products include pteropodine (positive modulators of muscarinic acid M) [6] coerulescine, horsfiline and elacomine (Fig. 1). Spirooxindole systems also constitute the core scaffold of many synthetic pharmaceuticals with a wide range of biological applications such as antimicrobial [7], antitumor [8], antibiotic [9] and inhibitors of the human NK-1 receptor [10].

In the course of our interest to develop nitrogen heterocyclic compounds [11] as anticancer agents, prompted us to investigate the biological activity of spirooxindole scaffold in detail. This called for a convenient and practical method for the synthesis of the spirooxindole unit. Literature survey reveals that 1,3-dipolar cycloaddition reactions have been used extensively to prepare spirooxindole derivatives [12]. The preparation of such compounds via the reaction of isatin and N-(2-aminophenyl)pyrrole at room temperature in the presence of different catalysts [13] such as molecular iodine, $ZnCl_2$, $CuCl_2$, $FeCl_3$, p-TSA, Sulfamic acid and HCl are also reported. In many cases the desired products were obtained in very low to moderate yields. Our efforts led to the development of a catalyst-free synthesis of pyrrole fused spirooxindoles in ethanol (Scheme 1).

The screening of these new spiro-heterocycles revealed that they possess significant cyctotoxicity and exhibit cyclin dependent kinase inhibition activities. Recently, spirooxindole derivatives (**B**)

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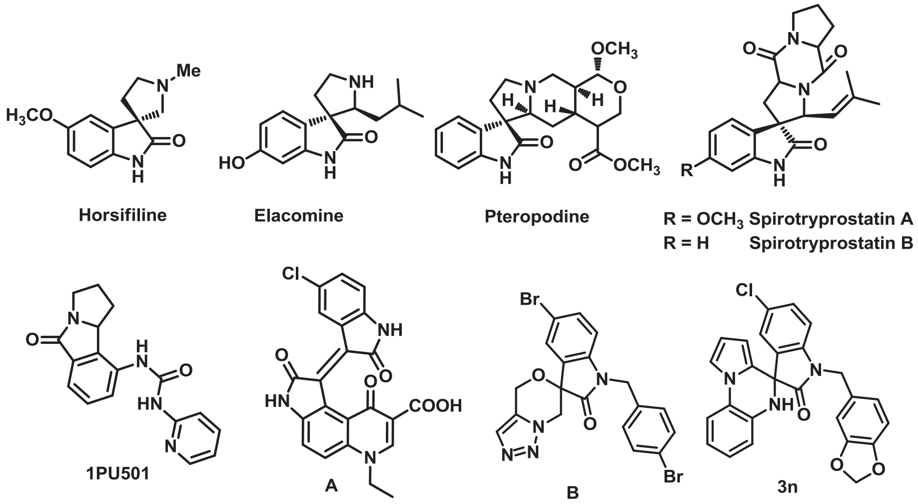
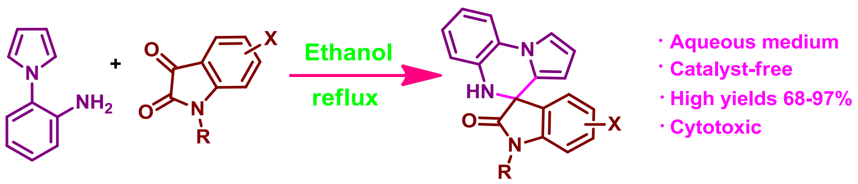


Fig. 1. Chemical structures of biologically active spirooxindole derivatives and CDK4 inhibitors.



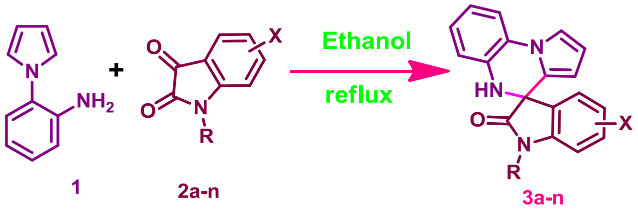
Scheme 1. Green synthesis of cytotoxic spirooxindole derivatives.

have been reported as apoptosis inducing scaffold which have emerged as an attractive synthetic target molecules [14], 1PU501 [15] (protein database code) and oxindole derivative [16] (**A**) depicted in Fig. 1, are the closest structural analogues of the title compounds that display CDK4 inhibitory activity, are presently in preclinical research for the treatment of human cancers. The results of our preliminary studies are presented in the following sections.

2. Results and discussion

2.1. Chemistry

Our studies began with exploring various non-catalytic routes for the cyclocondensation reaction between 2-(1H-pyrrol-1-yl)aniline (**1**) and isatin (**2**) (Scheme 2). Some of the representative reaction conditions that were investigated are depicted in Table 1. It was observed that this reaction did not proceed at all at room temperature when run neat or in water, acetonitrile, methanol or ethanol (entries 1–5). Interestingly, the product **3a** was obtained in 68% by heating the neat reaction at 100 °C for 12 h (entry 6). Subsequently it was revealed that the cyclocondensation proceeds efficiently in all the solvents tested under reflux temperatures (entries 7–10). Reaction in water afforded high yields of products (entry 7), however, recrystallization from ethanol was required for obtaining analytically pure samples. The reaction in refluxing ethanol, on the other hand gave a slightly higher yield (entry 10) and pure product crystallized on cooling the reaction mixture to room temperature. Considering the operational simplicity and lower temperature ethanol was preferred over water as the solvent.



Scheme 2. Synthesis of 5′H-spiro[indoline-3,4′-pyrrolo [1,2-a]quinoxalin]-2-one derivatives.

The scope and generality of the present protocol were then examined by employing various substituted isatins (Scheme 2) and the results are summarized in Table 2. The reaction tolerates both electron releasing as well as withdrawing substituents on the isatin component without any significant deviation in yields (entries **3b-i**). N-Protected isatins also afforded the corresponding products in excellent yields (entries **3j-n**). Importantly, analytically pure products are obtained by cooling, filtering and drying.

A plausible mechanism of a representative cyclocondensation to furnish **3a** may be advanced as shown in Scheme 3. Schiff base formation from the electron rich 2-pyrrolyl aniline **1** and the highly electrophilic isatin **2a** is followed by an intramolecular Friedel–Crafts type cyclization at the 2-position of the pyrrole. It may be recalled here that the intermediate **4a** has been prepared and independently converted to the product **3a** previously [13a].

2.2. Biological evaluation

2.2.1. Cytotoxic activity

All the newly synthesized compounds (**3a-n**) were evaluated for their cytotoxic activity in a panel of five human cancer cell lines, namely Hela (cervical), A549 (lung), DU-145 (prostate), HT-29 (colon) and MDA MB-231 (breast) by employing MTT assay [17], and doxorubicin was used as the reference drug. The results are summarized in Table 3 and expressed as IC_{50} values. The results revealed that some of these compounds exhibit promising