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Original article

Design and synthesis of pyrazole–oxindole conjugates targeting tubulin polymerization as new anticancer agents

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ABSTRACT

A series of twenty one compounds with pyrazole and oxindole conjugates were synthesized by Knoevenagel condensation and investigated for their antiproliferative activity on different human cancer cell lines. The conjugates are comprised of a four ring scaffold; the structural isomers **12b** and **12c** possess chloro-substitution in the D ring. Among the congeners **12b**, **12c**, and **12d** manifested significant cytotoxicity and inhibited tubulin assembly. Treatments with **12b**, **12c** and **12d** resulted in accumulation of cells in G2/M phase, disruption of microtubule network, and increase in cyclin B1 protein. Zebrafish screening revealed that **12b**, and **12d** caused developmental defects. Docking analysis demonstrated that the congeners occupy the colchicine binding pocket of tubulin.

1. Introduction

Microtubules, composed of αβ-tubulin heterodimers, are major constituents of the cytoskeleton in eukaryotic cells. The α- and β-tubulins are among the most highly conserved eukaryotic proteins [1]. Microtubules are pleiotropic in their function, particularly in organizing the spatial distribution of organelles in cells and chromosomes during cell division. Due to their essential functions in the cell, microtubules serve as an attractive drug target [2]. The dynamic equilibrium between microtubule polymerization and depolymerization is central to most of microtubule mediated functions including cell division [3]. This attribute has been exploited by numerous natural products and synthetic molecules which function as tubulin binding agents (TBAs) [4]. The microtubules posses three sites for ligand binding the vinca domain, colchicine domain and taxol domain. However, occurrence of peripheral neuropathy is a major limitation in development of antimicrotubule agents as drugs [5]. Therefore, discovery of novel molecules is required to overcome multi-drug resistance and neuropathies.

Oxindoles are important pharmacophores that are known to enhance anticancer activity of some established molecules. Sunitinib (**5**) and indirubin (**6**) are established anticancer agents containing oxindole as the basic scaffold (Fig. 1A) [6–8]. Recently, it has been observed that chromone–pyrimidine, chromone–indolinone, chromone–pyrazole, indole–pyrimidine, indole–indolinone and indole–pyrazole conjugates demonstrated profound growth inhibitory activity against different cancer cells [9]. Moreover substituted indolin-2-ones are potential inhibitors of p90 ribosomal S6 protein kinase [10]. In addition, tri and tetra substituted pyrazole derivates proved to have potent anticancer action due to the inhibition of p38α MAP kinase [11]. Whereas 4-arylazo-3,5-diamino-1*H*-pyrazoles function as novel group of ATP antagonists with moderate potency against CDK2–cyclin E complex [12]. The anticancer effects of some pyrazole amide derivatives are mediated by inhibition of the Elk-3 pathway and effects microtubules [13].

The unique feature of microtubule-binding agents, in contrast to other categories of anticancer drugs, is their incredible structural complexity and diversity, which provides many possibilities for

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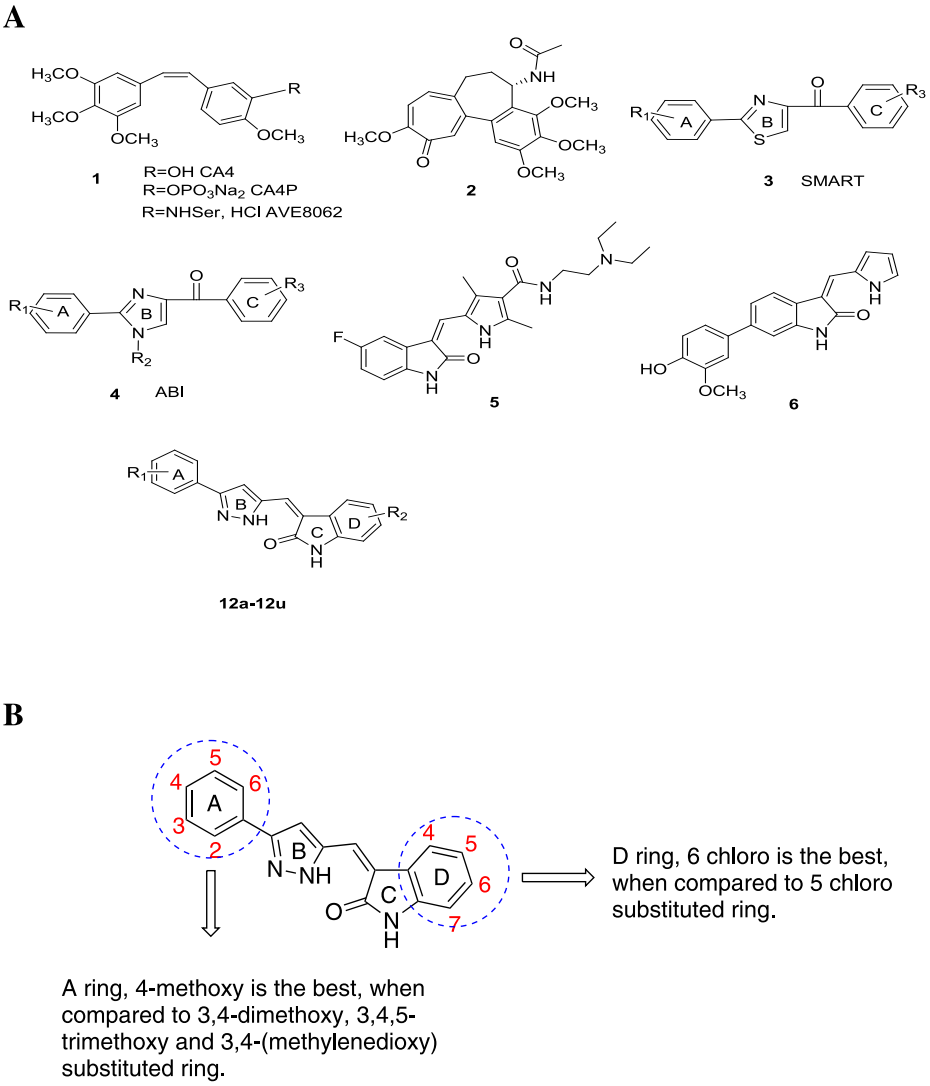

Fig. 1. A. Structures of some anticancer molecules Combretastatin (**1**), colchicine (**2**), substituted methoxylbenzoyl-aryl-thiazole (SMART) (**3**), 2-aryl-4-benzoyl-imidazole series (ABI) (**4**), Sunitinib (**5**), indirubin (**6**) and pyrazole–oxindole conjugates (**12a**–**u**). B. SAR of pyrazole–oxindole conjugates (see Table 1 for R_1, R_2).

optimization and new scaffold design. Combretastatin (**1**) and colchicine (**2**) possess a two ring and three ring scaffolds with a trimethoxyphenyl group for anchorage, which efficiently inhibit tubulin polymerization. Based on the ring geometry, the substituted methoxylbenzoyl-aryl-thiazole (SMART) (**3**) series contain a basic three ring scaffold and functions as tubulin inhibitors [14]. Due to limited water solubility, the core thiazole ring was modified to imidazole to generate 2-aryl-4-benzoyl-imidazole series (ABI) (**4**) (Fig. 1A) [15]. Nevertheless, the three ring (A, B and C) based scaffolds can be exploited further to generate better antitubulin molecules that could affect tubulin polymerization. Earlier we reported that oxindole derived imidazopyrazines exhibit significant anticancer activity [16]. In the present study, we performed the synthesis of (*Z*)-3-((3-phenyl-1*H*-pyrazol-5-yl) methylene) indolin-2-ones employing Knoevenagel condensation to generate a four ring scaffold (A: methoxy/methylene dioxy, B: pyrazole, C, D: oxindole). The congeners (**12a**–**u**) were functionalized at A ring and D ring with various substituents such as methoxy, methylene dioxy, chloro, fluoro and nitro groups (Fig. 1A).

2. Results and discussion

2.1. Chemistry

The Synthesis of (*Z*)-3-((3-phenyl-1*H*-pyrazol5yl)methylene) indolin-2-one analogs **12**(**a**–**u**) described in the study are outlined in Scheme 1. The final step has been carried out by means of Knoevenagel condensation between an equimolar mixture of oxindole/indolinone and 3-substituted phenyl-1*H*-pyrazole-5-carbaldehydes **11**(**a**–**d**) in the presence of piperdine in ethanol [6,9,10]. The key intermediates 3-substituted phenyl-1H-pyrazole-5-carbaldehydes **11**(**a**–**d**) was prepared in four sequential steps. Initially substituted acetophenones **7**(**a**–**d**) reacted with diethyl oxalate in the presence of sodium ethanolate in ethanol yielded ethyl 2,4-dioxo-4-(substituted phenyl)butanoates **8**(**a**–**d**). This was further cyclized with NH_2–$NH_2 \cdot 2HCl$ in ethanol to produce ethyl 3-substituted phenyl-1*H*-pyrazole-5-carboxylates **9**(**a**–**d**) in good yields [17,18]. The obtained carboxylates were reduced to (3-substitutedphenyl-1H-pyrazol-5-yl) methanols **10**(**a**–**d**) by $LiAlH_4$.