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Synthesis, biological evaluation and molecular docking studies of *trans*-indole-3-acrylamide derivatives, a new class of tubulin polymerization inhibitors

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ABSTRACT

In this study, we synthesized a series of *trans*-indole-3-acrylamide derivatives (**3a–k**) and investigated their activity for inhibition of cell proliferation against five human cancer cell lines (HeLa, MCF7, MDA-MB-231, Raji and HL-60) by MTT assay. Compound **3e** showed significant antiproliferative activity against both the Raji and HL-60 cell lines with IC_{50} values of 9.5 and 5.1 μM, respectively. Compound **3e** also exhibited moderate inhibitory activity on tubulin polymerization (IC_{50} = 17 μM). Flow cytometric analysis of cultured cells treated with **3e** also demonstrated that the compound caused cell cycle arrest at the G2/M phase in HL-60 and HeLa cells. Moreover, **3e**, the most active compound, caused an apoptotic cell death through the activation of caspase-3. Docking simulations suggested that **3e** binds to the colchicine site of tubulin.

1. Introduction

Cancer remains one of the leading causes of death worldwide and requires a pressing need for the development of novel and more effective treatments. Although the chemotherapy is the major method for treatment for various cancer types, the narrow dosing window of current drugs with regard to their efficacy and safety and significant drug resistance resulting a failure of antitumor drugs to exert their effects in certain cancer types limit the use of contemporary cancer chemotherapy. Therefore, the design and discovery of more effective and safer anticancer drug candidates are of interest in contemporary medicinal chemistry.[1,2]

Microtubules are important in mitosis and have been recognized as an important target for the development of novel anticancer drugs.[3] Agents targeting tubulin such as the vinca alkaloids and taxoids are potent chemotherapeutics currently used in the clinic. Among them, colchicine was the first tubulin-binding agent to have antivascular effects causing hemorrhagic necrosis in human tumors.[4] Combretastatins which are isolated from the South African tree *Combretum caffrum* are also a group of antimitotic compounds and combretastatin A-4 (CA-4, Fig. 1) is one of the well-known natural tubulin-binding molecule affecting microtubule dynamics.[5] CA-4 has provided researchers a simple structural template for the design of related compounds with potent activity and a large number of combretastatin analogues have been prepared as potential anticancer agents including chalcones, some of which have recently been reviewed.[6–10] Chalcones (1,3-diaryl-2-propen-1-ones) with an ionone system between two aromatic rings (Fig. 1) serve as precursors for the preparation of various flavonoids and exhibit interesting pharmacological activities[11,12] such as anticancer[13–15] and antiproliferative activities.[16–21] Their broad biological properties are reported to be due to the α,β-unsaturated ketone moiety.[22] Chalcones in which both the 1,3-diaryl rings are separated by α,β-unsaturated carbonyl system with three-carbon lengths are structurally similar to indolyl heterocycles (Fig. 1). There are many indole-based compounds found to

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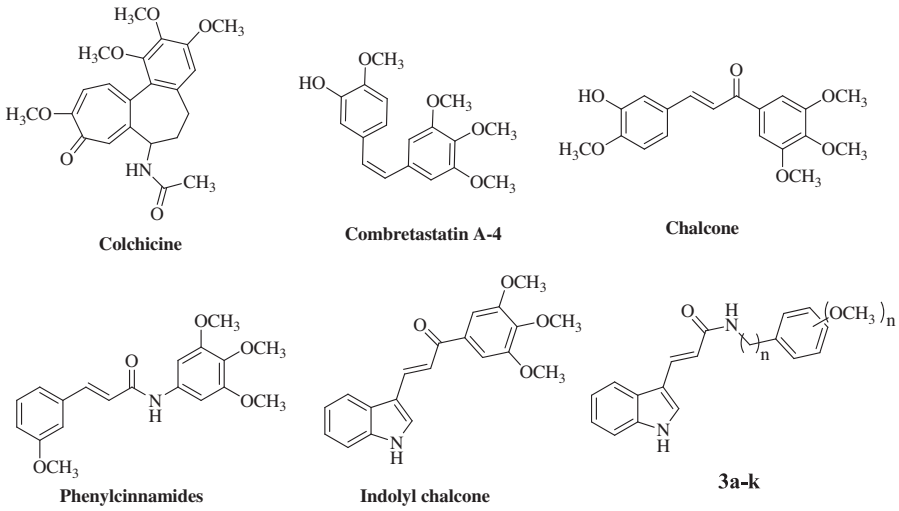


Figure 1. Chemical structures of known tubulin inhibitors and synthesized compounds.

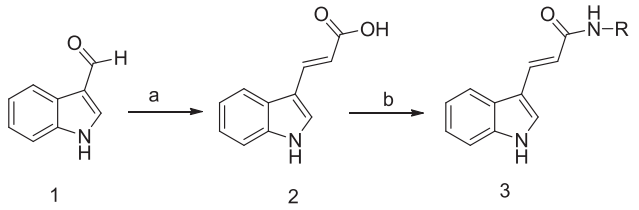
be effective as tubulin assembly inhibitors such as the recently reported 3-arylthioindoles, which induced significant apoptotic cell death.[23] However, indole-based chalcones remain largely unexplored for their anticancer potential.[24–26] Kumar et al. showed that indolyl chalcones inhibited the growth of A549, PaCa-2 and PC-3 cancer cell lines at micromolar concentrations.[27] Cinnamon amides having an α,β-unsaturated ketone moiety (phenylcinnamides in Fig. 1) are shown to bind to tubulin, thereby causing an inhibition of its polymerization and alteration in the tubulin-microtubule equilibrium.[28–31]

Encouraged with these results and to discover novel anticancer agents, we have synthesized a series of novel indolylacrylamide derivatives and evaluated their anticancer activities. The newly synthesized compounds structurally resemble the indolyl chalcone structure (Fig. 1).

2. Results and discussion

2.1. Chemistry

We synthesized a series of amide derivatives of *trans*-indole-3-acrylic acid as illustrated in Scheme 1. First, *trans*-indole-3-acrylic acid **2** was generated by Knoevenagel condensation of indole-3-carbaldehyde with malonic acid in the presence of piperidine as reported previously.[32] Treatment of **2** with appropriate amines in the presence of triethylamine and ethyl chloroformate, which was used as the carboxylate activator, produced *trans*-indole-3-acrylamide derivatives **3a**–**k** in moderate to good yields (40–69%). Compounds were purified by automated flash chromatography and checked for purity with UPLC before being tested in biological assays (purity was >97%). The structures of these compounds were confirmed by high resolution mass spectrometry (HRMS), IR and ^{1}H- and ^{13}C NMR spectral data. Final acryl amide derivatives exhibited a characteristic strong absorption peaks in the area of 1638–1733 cm^{-1}, which was attributable to the C=O of the amide moiety. In the ^{1}H NMR spectra of compounds **3a**–**k**, one of the olefinic protons (C*H*=CH–CO) was observed as a doublet at about 7.61–7.83 ppm, while the other (CH=C*H*–CO) was observed as a doublet at about 6.64–7.15 ppm, with coupling constants of 15.6 or 16.0 Hz indicating the presence of the (*E*) isomer.



Scheme 1. Reagent and conditions; (a) Malonic acid, piperidine, pyridine, 5 h, 40 °C; (b) 2, ethyl chloroformate, NEt_3, amine derivative, CH_2Cl_2, overnight, rt.

2.2. Biological evaluations

2.2.1. Effects of the compounds on the viability of cancer cells

trans-Indolyl-3-acrylamide derivatives **3a**–**k** were screened against five human cancer cell lines (HeLa, MCF7, MDA-MB-231, Raji and HL-60) using the 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT cell proliferation assay has been widely accepted as a reliable way to measure the cell proliferation rate when metabolic events lead to apoptosis or necrosis.[33–36] After incubation with compounds at different concentrations for 48 h, the cells were treated with MTT to measure their growth/viability (% of the untreated control) using a spectrophotometer as described previously.[37,38] Experiments were performed in quadruplicate. The IC_{50} values (Table 1) were calculated from concentration–response curves by means of the PRISM 5, GraphPad Software.[39]

In general, compounds having a phenylamidic moiety (**3a**–**e**) displayed greater antiproliferative potency than the molecules possessing a benzylamidic moiety (**3g**–**k**). Compounds having methoxy substituent at positions 3 and 4 or at 3 and 5 on the phenyl amidic moiety of the molecules (**3c**, **3d)** exerted potency on the Raji and HL-60 cell lines (IC_{50} values of 6.2-10.3 μM). Compound **3e** showed appreciable antiproliferative activity against both Raji and HL-60 cell lines, with IC_{50} values of 9.5 and 5.1 μM, respectively. Breast cancer cell lines MDA-MB-231 and MCF7 were not sensitive towards the newly synthesized compounds with the exception of the 3,4,5-trimethoxy-substituted phenyl amide derivative **3e**, which was the most effective compound found in the phenyl amide series.

In the series of benzylamide derivatives, compound **3h**, with 2,3-dimethoxy substitutions on the amide moiety, showed weak activity against HeLa cells. Derivatives **3g** (3-methoxy-4-hydroxy-substituted benzylamide derivative) and **3i** (2,5-dimethoxy-substituted benzylamide derivative) had weak activity against Raji cells.