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Design, synthesis of phenstatin/isocombretastatin-oxindole conjugates as antimitotic agents

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ABSTRACT

A series of phenstatin/isocombretastatin-oxindole conjugates was synthesized and tested for their cytotoxic activity against five human cancer cells such as prostate (DU-145), lung (A549), colon (HT-29), breast (MCF-7), liver (HepG2) cancer cells with IC_{50} values ranging from 0.049 to 38.90 μM. Amongst them, two conjugates (**5c** and **5d**) showed broad spectrum of antiproliferative efficacy on lung cancer cells with an IC_{50} value of 79 nM and 93 nM, respectively, whereas on colon cancer cells with an IC_{50} values 45 nM and 49 nM, respectively. In addition, cell cycle assay revealed that these conjugates (**5c** and **5d**) arrest at the G_2/M phase and leads to apoptotic cell death which was confirmed by Annexin V-FITC and mitochondrial membrane depolarization. Further, the tubulin polymerization assay analysis results suggest that these conjugates particularly **5c** and **5d** exhibit significant inhibitory effect on the tubulin assembly with an IC_{50} value of 1.23 μM and 1.01 μM, respectively. Molecular docking studies indicated that these compounds (**5c** and **5d**) occupy the colchicine binding site of the tubulin.

1. Introduction

Microtubules have become an important target in chemotherapy that are present in all eukaryotic cells and play an essential role in the formation of mitotic spindles, cell division etc.[1] The mitotic spindles are generated by noncovalent polymerization of α-tubulin and β-tubulin heterodimers that have become an attractive target to treat many types of malignancies.[2] The majority of drugs that binds at β-tubulin of the microtubule, are known to inhibit tubulin polymerization or depolymerization.[3] In particular, three major binding domains on β-tubulin subunit of microtubule, namely the vinca binding site, the taxane binding site and the colchicine binding site for mostly lipophilic ligands and some high affinity agents have been developed as antiproliferative drugs.[3,4] More particularly, compounds such as the paclitaxel, docetaxal and epothilones, that bind to the taxane binding site prevent microtubule disassembly by stabilizing microtubules and are used in the treatment of carcinomas, such as lung, breast, ovarian, and bladder.[5] In contrast, compounds like colchicinium, vinca alkaloids and combretastatin A-4 (**1**, Fig. 1) and nocodazole, that bind to the vinca or colchicine binding sites inhibit cancer cell proliferation and tubulin assembly by destabilizing microtubules and are used in the treatment of leukemia, lymphoma and a variety of other diseases.[6] However, many of such agents manifest different limitations in their clinical utility, therefore development of new microtubule targeting agents is of significance.

Phenstatin (**2a**, Fig. 1) is a benzophenone motif reported by Pettit and coworkers and it is also well known microtubule-destabilizing agent. Phenstatin (**2a**, Fig. 1) and its derivatives exhibits the most potent activity at the colchicine binding site and significantly inhibits tubulin polymerization and exerts profound antiproliferative activity against various human cancer cell lines, including multidrug-resistant cancer cells.[7] Recently, a new class of benzophenone series with insertion of small heterocyclic groups in the B-ring like indole, quinoline, carbazole, thiophenes displayed excellent cytotoxic activity as well as significant inhibition of tubulin polymerization.[8] Recently, isocombretastatin A-4 (iso CA-4, **2c**) (Fig. 1) is a non isomerized C A-4, reported as antimitotic agent. In addition, isocombretastatin and its derivatives shows substantial cytotoxic activity towards selected human cancer cell lines and cells are accumulated in the G2/M phase of the cell cycle.[9]

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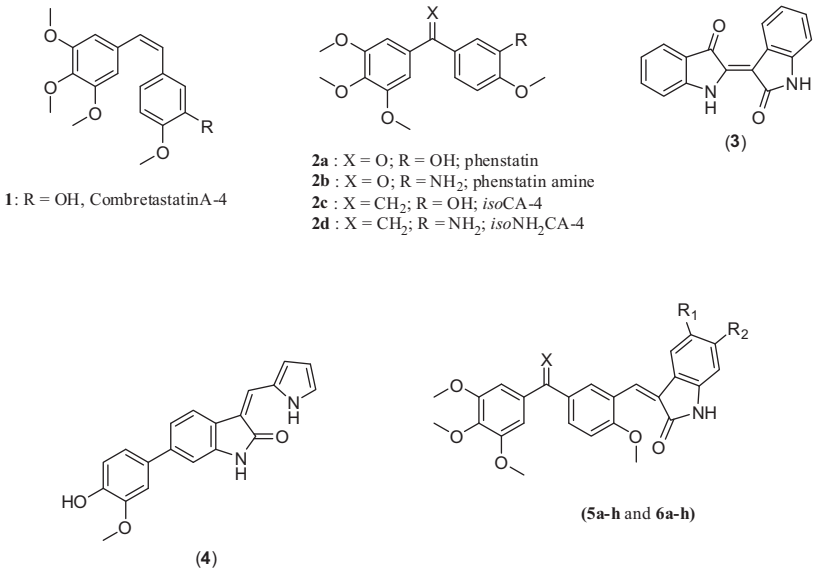

Figure 1. Chemical structures of microtubule targeting agents. Combretastatin A-4 (**1**), phenstatin (**2a**) phenstatin amine (**2b**) isocombretastatin A-4 (**2c**) isocombretastatin A-4 amine (**2d**), indirubin (**3**), A-432411 (**4**) phenstatin/isocombretastatin-oxindole conjugates (**5a**–**h** and **6a**–**h**).

Oxindoles are versatile moieties that display diverse biological activities, including anticancer activity. Bis-indole alkaloid indirubin (**3**, Fig. 1) is interesting natural pharmacophore and traditional Chinese medicine recipe used in the treatment of leukemias. This has broad spectrum of activity and it is mainly recognized as kinase inhibitor. In addition, indirubins are potent inhibitors of cyclin-dependent kinases (CDKs) like glycogen synthase kinase-3b (GSK-3b) and CDK1/cyclin B.[10] A-432411 (**4**, (*Z*)-3-((1*H*-pyrrol-2-yl)methylene)-6-(4-hydroxy-3-methoxyphenyl)indolin-2-one) (Fig. 1) is an indolinone that is structurally different from other known synthetic microtubule inhibitors. This compound is efficacious against a variety of human cancer cell lines including drug-resistant HCT-15 that over expresses Pgp170. These compete with the colchicine-binding site on the tubulin thereby inhibiting microtubule polymerization and causing G2–M arrest and induces apoptosis.[11]

Thus based on these observations, it was considered of interest to explore newer antitubulin agents by linking different pharmacophores. In this study, we linked the substituted oxindoles with phenstatin and isocombretastatin A-4 to generate phenstatin/isocombretastatin A-4 oxindoles. The promising activity observed prompted us to investigate the role of these new compounds in the proliferation and apoptosis of human colon cancer cell line (HT-29). We also investigated the effect of these compounds on proteins that regulate cell-cycle progression.

2. Results and discussion

2.1. Chemistry

These phenstatin/isocombretastatin-oxindole conjugates (**5a**–**h** and **6a**–**h**) were prepared employing Knoevenagel reaction between equimolar mixtures of substituted oxindoles (**17a**–**h**) and phenstatin 3-aldehyde/isocombretastatin 3-aldehyde (**16a**–**b**) in the presence of piperidine as shown in Scheme 1. The compound structures were confirmed by means of ^{1}H NMR, ^{13}C NMR, HRMS and IR spectra.

The phenstatin 3-aldehyde/isocombretastatin 3-aldehyde (**16a**–**b**) were obtained via Swern's oxidation of the corresponding (3-(hydroxymethyl)-4-methoxyphenyl)(3,4,5-trimethoxyphenyl) methanone (**15a**) and (2-methoxy-5-(1-(3,4,5-trimethoxyphenyl) vinyl)phenyl)methanol (**15b**). The ketone **13** is generated from the benzhydrol derivative **12** via oxidation and subsequent Witting methylation afforded the olefin **14**. The benzhydrol derivative **12** was in turn, obtained via the addition of the aryl lithium reagent generated from the iodide **10** to aldehyde **11**. The aryl iodide **10** was obtained via a three step synthetic manipulation of 5-iodo salicylic acid **7** (Scheme 1).

2.2. Biological studies

2.2.1. Antiproliferative activity

In an attempt to examine the structure activity relationship of phenstatin/isocombretastatin-oxindole conjugates (**5a**–**h** and **6a**–**h**) consisting A, B, C, D-rings and X (Fig. 2) as shown in Scheme 1. Sixteen compounds were prepared with respect to different modifications made on the D-ring and X which were evaluated for cytotoxic activity against a panel of five human cancer cell lines such as A549 (lung), DU-145 (prostate cancer), HT-29 (colon cancer), MCF-7 (breast cancer) and HepG2 (liver cancer) by employing MTT assay.[12] Combretastatin A-4 was used as reference drug and the results are summarized in Table 1 and expressed as IC_{50} values.

The in vitro screening results revealed that these conjugates possess excellent to moderate cytotoxic activity with IC_{50} values ranging from 0.049 to 38.90 μM. Particularly, the phenstatin-oxindole conjugates harboring carbonyl group in between A and B rings showed profound cytotoxic activity with an IC_{50} value in the range 0.045–35.55 μM. More particularly, the conjugate 5**c** having electron donating substituent like *para*-methoxy group on D-ring possess excellent cytotoxicity against lung and colon cancer cells with IC_{50} value 79 nM and 45 nM, respectively. In contrast, substitution with nitro at para position of D-ring as in **5b** proved deleterious for its antiproliferative activity against human prostate cancer like Du145 cells (IC_{50} value is 35.55 μM). However, isocombretastatin-oxindole conjugates (**6a**–**h**) harboring ethylene group in between A and B ring displayed potent antiproliferative activity with an IC_{50} value in the range 0.081–38.9 μM. In addition, the conjugate **6c** having *para*-methoxy group on D-ring as electron donating substituent exhibited significant cytotoxic activity towards colon cancer (HT29) with an IC_{50} value 81 nM.