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Synthesis of different heterocycles-linked chalcone conjugates as cytotoxic agents and tubulin polymerization inhibitors

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ABSTRACT

A series of new heterocycles-linked chalcone conjugates has been designed and synthesized by varying different alkane spacers. These conjugates were tested for their *in vitro* cytotoxic potential against a panel of selected human cancer cell lines namely, lung (A549 and NCI-H460), prostate (DU-145 and PC-3), colon (HCT-15 and HCT-116), and brain (U-87 glioblastoma) by MTT assay. Notably, among all the tested compounds, **4a** exhibited potent cytotoxicity on NCI-H460 (lung cancer) cells with IC_{50} of 1.48 ± 0.19 μM. The compound **4a** showed significant inhibition of tubulin polymerization and disruption of the formation of microtubules (IC_{50} of 9.66 ± 0.06 μM). Moreover, phase contrast microscopy and DAPI staining studies indicated that compound **4a** can induce apoptosis in NCI-H460 cells. Further, the flow-cytometry analysis revealed that compound **4a** arrests NCI-H460 cells in the G2/M phase of the cell cycle. In addition, molecular docking studies of the most active compounds **4a** and **4b** into the colchicine site of the tubulin, revealed the possible mode of interaction by these new conjugates.

1. Introduction

Tubulin is an α,β-heterodimeric protein that forms the cytoskeletal framework of microtubules.[1] It is one of the most active molecular targets of numerous anticancer ligands that cause cell cycle arrest at the G2/M phase by interfering with the microtubule polymerization/depolymerization process.[2,3] Many clinically used natural product anticancer agents such as colchicines (**A**, Fig. 1), MDL-27048, taxanes, combretastatin A-4 (**B**), podophyllotoxin and vinca alkaloids are tubulin-binding agents.[4] Antimitotic agents generally interact with tubulin at three known binding sites namely, the colchicine, the vinca alkaloid and the paclitaxel binding sites. Agents that target the colchicine site (e.g., colchicines, combretastatin A-4 and podophyllotoxin)[5] or the vinca alkaloid site (e.g., vinblastine)[6] are known as microtubule destabilizing agents. On the other hand, agents that bind to the paclitaxel site (e.g., paclitaxel)[7] are referred to as microtubule-stabilizing agents. As microtubules play a vital role in several cellular functions such as the formation of the mitotic spindle, cytoplasmic organelle movement, maintaining cell shape, intra-cellular transport and cell replication, interfering with microtubule dynamics lead to severe side effects.[8–10] Therefore, it is essential to develop new tubulin-binding agents and antimitotic agents with novel modes of action to overcome side effects as well as drug resistance.

Chalcones (1,3-diaryl-2-propen-1-ones) are precursors of flavonoids and isoflavonoids that possess a wide range of biological activities such as anticancer, anti-diabetic, anti-hypertensive, anti-viral, anti-inflammatory, anti-tuberculosis, anti-oxidant, anti-leishmanial, anti-filarial, anti-malarial, anti-bacterial and anti-fungal activities.[11–14] The biological activities of chalcones are largely attributed to the presence of α,β-unsaturated ketone functionality, as removal of this group makes them inactive. The extensive exploration of chalcones can be attributed to their ease of synthesis, relatively simpler chemical architecture, being precursors for important synthetic manipulations and promising biological activities. Naturally occurring chalcones and their synthetic analogues displayed significant cytotoxic activity against various cancer cells.[15] One of the most widely proposed anticancer mechanisms of chalcones is the prevention of tubulin polymerization by binding to the colchicine-binding site.[16–18] Due to their promising anti-cancer activities, considerable efforts have been dedicated to discovering new potential chalcone-based drug candidates during the last decade. A majority of these naturally occurring anti-cancer compounds are substituted with electron donating hydroxy and/or

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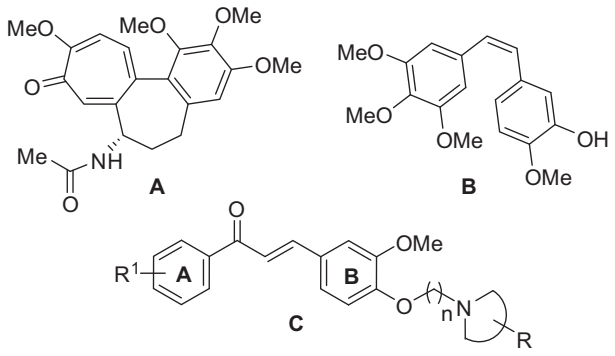


Fig. 1. The structures of colchicine (**A**), combretastatin A-4 (**B**), and heterocycles-linked new chalcone conjugates **4a**–**y** (**C**).

methoxy groups at various positions around the chalcone scaffold.[19,20] In order to ascertain more advanced structure–activity relationships (SARs) of chalcones, it is of great importance to synthesize new compounds with more diverse substitutions patterns.

In continuation of our earlier efforts in the field of anti-cancer drug discovery,[21–25] herein, we report the synthesis of a series of novel heterocycles-linked chalcone conjugates (**C**, Fig. 1) with diverse substitutions and evaluated for their *in vitro* cytotoxic activity on selected human cancer cell lines.

2. Results and discussion

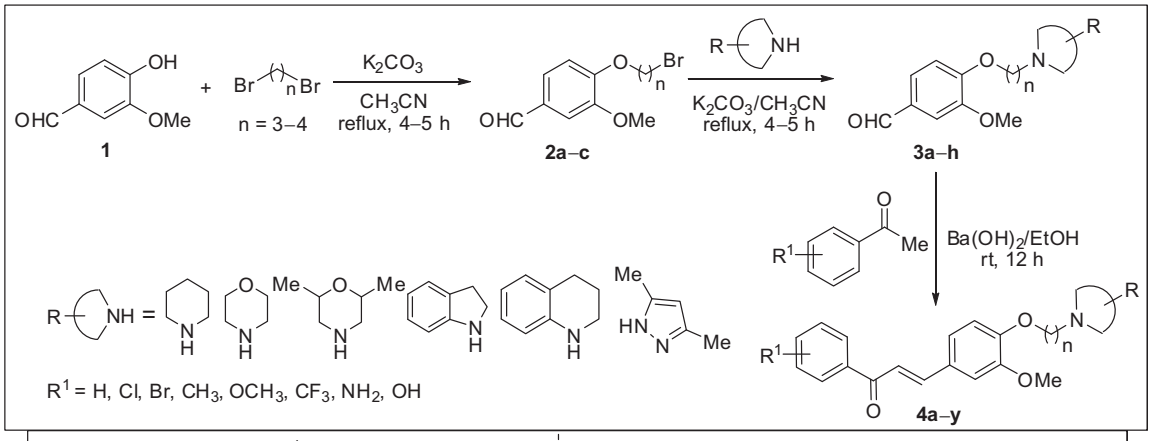
2.1. Chemistry

Two core structural components (i) an aldehyde, and (ii) a ketone are required for achieving the target chalcone conjugates **4a**–**y**. The synthetic route for the preparation of the heterocycles-linked chalcone conjugates **4a**–**y** was outlined in Scheme 1. Initially, the etherification of vanillin (**1**) was carried out with dibromo alkane spacers of varying lengths (n = 2, 3, 4), in the presence of K_2CO_3 as the base to give the intermediates **2a**–**c**. Next, various nitrogen containing heterocycles were tethered to the alkane spacer of **2** by refluxing in acetonitrile in the presence of K_2CO_3, to afford the aldehyde intermediates **3a**–**h** in quantitative yields. Finally, aldol condensation reaction was performed between intermediates **3a**–**h** and a variety of substituted acetophenones by using $Ba(OH)_2$ as the base to furnish the chalcone derivatives **4a**–**y** in good yields. All the newly synthesized compounds were characterized by HRMS, ^1H and ^{13}C NMR spectroscopy. Almost similar pattern was observed in ^1H and ^{13}C NMR spectra of all the final synthesized compounds of this series. The double bond of the chalcone moiety was in the *trans*-configuration as denoted by the coupling constant (*J*) values of 15–16 Hz. In the ^{13}C NMR spectrum, the characteristic peaks corresponding to the chalcone moiety were observed: the carbonyl carbon appeared in the range δ 183–192 whereas the aromatic carbons along with the olefinic carbons appeared in the range of δ 163–104. The HRMS (ESI) of all the compounds showed an $[M+H]^+$ peak equivalent to their molecular formulae.

2.2. Biological evaluation

2.2.1. In vitro cell growth inhibitory activity

The new chalcone conjugates **4a**–**y** were evaluated for their *in vitro* antiproliferative activity against a panel of seven human cancer cell lines namely, lung (A549 and NCI-H460), prostate (DU-145 and PC-3), colon (HCT-15 and HCT-116) and brain (U-87 glioblastoma) by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Concentration-response course



4a; R = piperidinyl, n = 3, R^1 = 4-methoxy
4b; R = piperidinyl, n = 3, R^1 = 2-hydroxy
4c; R = piperidinyl, n = 3, R^1 = 4-bromo
4d; R = piperidinyl, n = 4, R^1 = H
4e; R = piperidinyl, n = 4, R^1 = 4-bromo
4f; R = piperidinyl, n = 4, R^1 = 4-chloro
4g; R = piperidinyl, n = 4, R^1 = 2,4-dichloro
4h; R = morpholinyl, n = 2, R^1 = 4-methoxy
4i; R = morpholinyl, n = 2, R^1 = 4-bromo
4j; R = morpholinyl, n = 2, R^1 = 2,4-dichloro
4k; R = morpholinyl, n = 2, R^1 = 4-trifluoromethyl
4l; R = morpholinyl, n = 3, R^1 = 4-methoxy
4m; R = 2,6-dimethylmorpholinyl, n = 3, R^1 = 4-methoxy
4n; R = 2,6-dimethylmorpholinyl, n = 3, R^1 = 2-methoxy
4o; R = 2,6-dimethylmorpholinyl, n = 3, R^1 = 2,4-dichloro
4p; R = 2,6-dimethylmorpholinyl, n = 3, R^1 = 4-methyl
4q; R = indolinyl, n = 3, R^1 = 4-methoxy
4r; R = indolinyl, n = 3, R^1 = 2,4-dichloro
4s; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R^1 = H
4t; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R^1 = 4-methoxy
4u; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R^1 = 4-bromo
4v; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R^1 = 4-chloro
4w; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R^1 = 2,4-dichloro
4x; R = 1,2,3,4-tetrahydroquinolinyl, n = 3, R^1 = 4-amino
4y; R = 3,5-dimethyl-1*H*-pyrazolyl, n = 3, R^1 = 4-methoxy

Scheme 1. Synthesis of different heterocycles-linked new chalcone conjugates **4a**–**y**.