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Design, synthesis and biological evaluation of Novel Curcumin Analogs with anticipated anticancer activity

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ABSTRACT

Context: Extensive research conducted within past years revealed that curcumin is a highly pleiotropic molecule that interacts with a diverse range of molecular targets and hence it possess anti-proliferative activities against tumor cells.The great similarities between curcumin analogs and chalcones inspired their testing against tubulin enzyme activity as recent research revealed that chalcones possess cytotoxic activity associated with tubulin inhibition and interference with microtubule formation, which is essential in mitosis and cell replication.
Objective: Novel Curcumin analogs were designed, synthesized and tested for their antitumor activities. Also in silico and in vitro studies has been performed to predict the binding affinity of the target compounds and to test their ability to inhibit tubulin assembly and act as microtubule destabilizing agents.
Methods: Six novel curcumin analogs were designed & synthesized with 3,5-dibenzylidenepiperidin-4-one core moiety.
Results: Compounds showed interaction energy comparable to or within the range of podophyllotoxin itself when docked into the colchicine binding site of tubulin using the podophyllotoxin-tubulin complex (PDB 1SA1).
Conclusion: Compounds showed moderate anticancer activity and moderate ability to destabilize microtubules and thus inhibiting tubulin polymerization, as a result; these compounds could be used for further future development to obtain more potent analogs.

1. Introduction

Cancer is a major concern nowadays for research, although a range of therapies based on chemotherapy are available, but they are of limited efficacy or of serious adverse effects and associated with high levels of toxicity. Hence, many research are being performed for the aim to develop new chemotherapies with minimal side effects on mammalian cells. That was reachable through natural product compounds which were found to be a good source for both novel and potent bioactive compounds with minimal side effects in vivo [1–10].

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione] Fig. 1, is a turmeric powder, extracted from the rhizome of the plant curcuma longa. Curcumin was found to be a highly pleiotropic molecule that interacts with a diverse range of molecular targets and hence it possess anti-proliferative activities against tumor cells in vitro, anti-inflammatory, antibacterial, antiviral and anti-hepatotoxic activities [11–14]. Since cancer is a result of the dys-regulation of multiple cell signaling pathways so curcumin's multi-targeting ability may be the key to its therapeutic potential against cancer. On the other hand, recent research revealed that chalcones possess cytotoxic activity, where tubulin inhibition and interference with microtubule formation were believed to be the main reason for this cytotoxicity; tubulin protein and microtubule formation are essential in cellular processes such as mitosis and cell replication and hence, the great similarity in structure between curcumin analogs and chalcones inspired their testing against tubulin activity [15].

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2. Results and discussion

2.1. Chemistry

Analogs **III b, IV a, IV b, and VII a–c** represent two different series of compounds designed and synthesized in order to have the *3,5-dibenzylidenepiperidin-4-one* core moiety. The synthesis of series 1 involves three reactions; first, the unisolated compounds **II a, b** were prepared by acylation of **I a** with the appropiateacyl chloride in the presence of triethylamine. In the next step these derivatives **II a, b** were then condensed with N-piperidone.HCl in the presence of acidic medium to get products **III a, b** after neutralization. The separation of the product **III a** was quite difficult as it was insoluble. Finally these derivatives **III a, b** undergo acylation or benzoylation in presence of triethylamine, then purification and crystallization were performed to obtain pure final compounds **IV a–b** (Scheme 1).

Series 2 was obtained first by activation of hydroxy groups of **I a, b** by reflux with sodium metal followed by alkylation with propyl chloride. The obtained unseparated products **V a, b** were then condensed with N-piperidone.HCl in presence of acidic medium followed by neutralization to produce the unseparated compounds **VI a, b** after neutralization which were then acylated in presence of triethylamine or reacted with phenylisothiocyanate in presence of triethylamine and reflux, then products were purified and crystallized to obtain pure **VII a–c** (Scheme 2).

2.2. Molecular modeling study

All the newly synthesized analogs were docked into the colchicine binding site of tubulin using the podophyllotoxin-tubulin complex (PDB 1SA1) as template [16,17]. In this study we observed that the synthesized analogs (**IV a, b and VII a–c**) showed interaction energy comparable to or within the range of podophyllotoxin itself, this reveals the ability of these compounds to inhibit tubulin assembly and act as microtubule destabilizing agents. Podophyllotoxin binds into a hydrophobic pocket on b-tubulin that contains two hydrophobic centers; one surrounded by Metb259, Alab316, and Lysb352 (occupied by the benzodioxole fragment in podophyllotoxin), and the other surrounded by Leub242, Alab250, and Leub255 (occupied by the trimethoxyphenyl moiety) [18] Fig. 2. Novel compounds occupy the same hydrophobic pocket with a methoxy group and oxygen atom of piperidone ring surrounded by Leub242, Alab250 & Leub255 and acetyl group surrounded by Metb259, Alab316 & Lysb352, some compounds also make an extra binding through H-bond either or Π-bond Table 1.

2.3. Biological evaluation

2.3.1. Anti-tumor properties

The synthesized compounds **IV b, VII b and VII c** were screened for their anti-tumor properties against; ovarian cancer (A2780), renal adenocarcinoma (ACHN), prostate cancer (PC-3), colorectal cancer (Hct-116) and a leukemic monocyte lymphoma (U937-GTB) utilizing the Fluorometric Microculture Cytotoxicity Assay FMCA method. Those compounds exhibited considerable anti-tumor activity against the five cell lines used in the assay with (IC_{50}, concentration required to produce 50% inhibition of cell growth compared to control experimental) ranging from 190.14 to 246.45 μM as shown in Table 2 and Graph 1–3.

2.3.2. Tubulin polymerization assay

The Tubulin polymerization activity of the compounds **IV b**, **VII b** and **VII c** are summarized in Table 3 and Graph 4. From the results; compounds **IV b and VII c** showed mild to moderate microtubule assembly inhibition in this assay, where compound **VII b** was the most potent among the tested compounds in destabilizing microtubules compared to both positive control drugs; "Vincristine as an example of microtubule destabilizing agent and Docetaxel as an example of microtubule stabilizing agent [19], which is correlated to the docking interaction results obtained in the previous silico study.

3. Conclusion

The novel synthesized analogs proved to have moderate anticancer activity and moderate ability to destabilize microtubules and thus inhibiting tubulin polymerization and forcing apoptosis of the cancer cells, which can make them promising leads for further investigation in the future by their development.

4. Experimental

4.1. Chemistry

Starting materials and reagents were purchased from Sigma–Aldrich. Melting points were recorded on Stuart Scientific apparatus.^{1}H NMR spectra were recorded on a varian Mercury VX-300 NMR spectrometer. MS spectra mass were recorded on Shimadzu GCMS-QP 5050A gas chromatograph mass spectrometer (70 $_e$V). Elemental analysis was performed at The Regional Center for Mycology and Biotechnology Al-Azhar University.

4.1.1. 3-Ethoxy-4-propanoyloxybenzaldehyde (II a) [20]

A solution of ethyl vanillin in chloroform was added to propionyl chloride in equimolar manner at room temperature followed by 1 h reflux and the resulting precipitate was isolated by filtration, washed with water, and dried, giving (84%) yield of **"II a"**; mp = 107–108 °C as reported.

4.1.2. 4-Butanoyloxy-3-ethoxybenzaldehyde (II b) [20]

A solution of ethyl vanillin in chloroform was added to butyryl chloride in equimolar manner at room temperature followed by 1 h reflux and the resulting precipitate was isolated by filtration, washed with water, and dried, giving (92%) yield of **"II b"**; mp = 110–111 °C as reported.

4.1.3. 3, 5-bis (4-Butyryloxy-3-ethoxybenzylidene) piperidin-4-one (III b)

1.5 mol of butyryl chloride and 3 mol of triethylamine were dissolved together in chloroform and added dropwise at 5 °C to solution of 1 mol of ethyl vanillin **"I a"** in chloroform then stirring

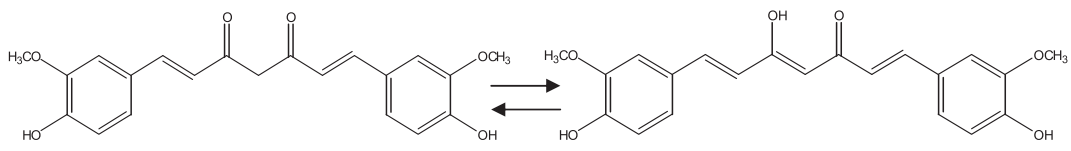

Fig. 1. Curcumin structure.