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# Design, synthesis and biological evaluation of millepachine derivatives as a new class of tubulin polymerization inhibitors

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ABSTRACT

A series of novel tubulin polymerization inhibitors (**9a**–**9p**) have been synthesized and evaluated for their in vitro and in vivo biological activities. Among these compounds, **9e** displayed strong antiproliferative activity against several tumor cell lines ($IC_{50}$ = 0.15–0.62 μM). Compound **9e** was also shown to arrest cells in the G2/M phase of the cell cycle and inhibit the polymerization of tubulin. Molecular docking studies suggested that **9e** binds into the colchicine binding site of tubulin. In xenograft experiments, **9e** exerted more potent anticancer effect than anticancer drug taxol against the H460 Human lung carcinoma in BALB/c nude mice. In summary, these findings suggest that **9e** is a promising new antimitotic compound for the potential treatment of cancer.



## 1. Introduction

Microtubules, composed of α/β-tubulin heterodimers, are major components of the cytoskeleton of eukaryotic cells and play an important role in a wild number of fundamental cellar functions, such cell division, formation, maintenance of cell shape, regulation of motility, cell signaling, secretion, and intracellular transport.[1–5] The formation of microtubules is a dynamic process that involves the polymerization and depolymerization of α and β tubulin heterodimers.[1] Disruption of the dynamic equilibrium blocks the cell division machinery at mitosis and leads to an increase in the number of cells in metaphase arrest, resulting in cell death.[6,7] Given their significant role in the growth and function of cells, the microtubule has become an important target for the design and development of new antimitotic anticancer agents.[8,9]

Antimitotic agents are generally derived from natural products and fall into two classes: (1) microtubule stabilizers including taxoids (e.g., paclitaxel and docetaxel)[10] along with the more recently discovered epothilones,[11] discodermolide,[12] and eleutherobin,[13] which prevent the depolymerization of tubulin; and (2) microtubule disrupters, such as Vinca alkaloids (e.g., vincristine, vinblastine),[14] colchicines,[15] podophyllotoxin,[16] and combretastatin A-4,[17] which inhibit the polymerization of tubulin (Fig. 1). Nevertheless, clinical use of these compounds is facing severe disadvantages, including high toxicity, marginal oral bioavailability, poor solubility, complex synthesis, and drug resistance.[18,19] Therefore, synthetic low molecular weight compounds with high oral bioavailability and potent antitumor activity for first and second line therapy are urgently needed.

Chalcones are naturally occurring compounds acting as precursors in the biosynthesis of flavonoids and isoflavonoids, which are an important pharmacophore of various natural products. Chalcones also display a variety of biological activities, such as antimitotic,[20] antiproliferative,[21] anti-inflammatory,[22] antimalarial,[23] anti-bacterial,[24] and anti-fungal activities.[25] Over the last few years, the development of chalcones as tubulin-binding agents has been drawn widely attention. Recent studies have shown that some compounds containing the chalcone skeleton act as cytotoxic agents or as microtubule destabilizing agents, targeting the colchicine binding site.[20,26–28]

Millepachine, one of naturally occurring pyranochalcones, is isolated from *Millettia pachycarpa* for the first time in our group, exhibited a potent apoptosis inducing effects, being a promised candidate for the treatment of cancer.[29,30] As previously reported,[31] we designed and synthesized a series of millepachine derivatives and evaluated for their in vitro and in vivo antiproliferative activity as well as tubulin polymerization inhibitory activity. Among these novel derivatives, compound **1** was identified as a potent anti-tumor agent which is a potential tubulin polymerization inhibitor. Unfortunately, the poor solubility and low bioavailability

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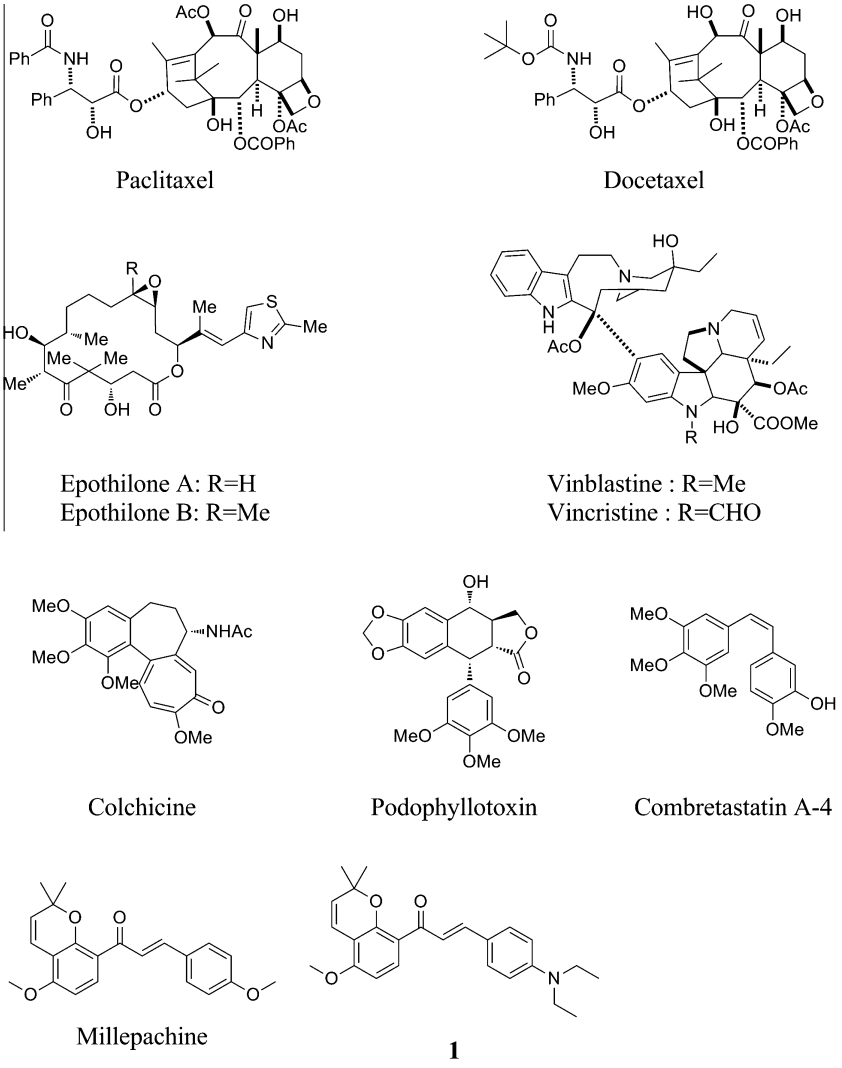


**Figure 1.** Examples of antimitotic natural products.

(7.08%) of compound **1** possibly limited the druggability. Our goal was to discover an orally available, low toxicity, tubulin inhibitor. Therefore, as a consequence of the structural optimization, compound **1** was taken for further modification in order to get better antiproliferative activity and oral bioavailability.

Herein, we report the synthesis and bioactivities of a series of substituted pyranochalcone compounds based on lead compound **1**. The synthesized compounds were evaluated for their cytotoxic activity toward five different human cancer cell lines and one normal human cell line. Among these derivatives, compound **9e** exhibited most potent antiproliferative activity against HepG2, A549, A375, SMMC-7221 and K562 cells ($IC_{50}$ = 0.15, 0.36, 0.62, 0.61 and 0.52 μM, respectively). Compound **9e** was also investigated for its inhibition of tubulin polymerization activity. In addition, computer-based docking studies have been performed to understand the interaction between tubulin and small molecule inhibitors.

## 2. Chemistry

The synthetic pathway to achieve title compounds **9a**–**9p** is shown in Scheme 1. 2,4-Dihydroxyacetophenone **2** was regioselectively monoprotected on the nonhydrogen-bonded phenol using $K_2CO_3$ in dry acetone and methoxymethylene chloride (MOMCl) at room temperature to provide **3**. Treatment of **3** with 3-chloro-3-methyl-1-butyne and NaH in anhydrous DMF to afford compound **4** which heating in pyridine at 120 °C for overnight, underwent Claisen rearrangement, leading to compound **5**. Condensation of **5** and 4-(diethylamino)benzaldehyde **6** under Claisen–Schmidt conditions using 50% KOH in methanol provided the desired compound **7**. Deprotection of the MOM groups using HCl in ethyl acetate at room temperature yielded key intermediate **8**, followed by an esterification or etherification of the liberated phenol with diethyl sulfate, alkyl halide, acid anhydride, sulfonyl chloride and acid to afford the final desired products **9a**–**9p**.

## 3. Biological results and discussion

### 3.1. In vitro antiproliferative activities

Compounds **9a**–**9p** were evaluated for in vitro cytotoxic activity against five human cancer cell lines, including human hepatocellular carcinoma (HepG2), human lung adenocarcinoma (A549), human malignant melanoma (A375), human hepatocellular carcinoma (SMMC-7221), human chronic myelogenous leukemia (K562) and one normal human cell line (LO2). The results expressed as $IC_{50}$ values, are reported in Table 1. It was notable that compound **9e**, **9l**, and **9n** exhibited stronger cytotoxicity activity