Biochemical Pharmacology 88 (2014) 291–302

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

PM060184, a new tubulin binding agent with potent antitumor activity including P-glycoprotein over-expressing tumors

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ARTICLE INFO

Article history:
Received 29 November 2013
Accepted 22 January 2014
Available online 31 January 2014

Keywords:
Microtubules
Tubulin modulators
Polymerization inhibitors
Antimitotic agents
P-glycoprotein
Cancer therapy

ABSTRACT

PM060184 belongs to a new family of tubulin-binding agents originally isolated from the marine sponge *Lithoplocamia lithistoides*. This compound is currently produced by total synthesis and is under evaluation in clinical studies in patients with advanced cancer diseases. It was recently published that PM060184 presents the highest known affinities among tubulin-binding agents, and that it targets tubulin dimers at a new binding site. Here, we show that PM060184 has a potent antitumor activity in a panel of different tumor xenograft models. Moreover, PM060184 is able to overcome P-gp mediated resistance *in vivo*, an effect that could be related to its high binding affinity for tubulin. To gain insight into the mechanism responsible of the observed antitumor activity, we have characterized its molecular and cellular effects. We have observed that PM060184 is an inhibitor of tubulin polymerization that reduces microtubule dynamicity in cells by 59%. Interestingly, PM060184 suppresses microtubule shortening and growing at a similar extent. This action affects cells in interphase and mitosis. In the first case, the compound induces a disorganization and fragmentation of the microtubule network and the inhibition of cell migration. In the second case, it induces the appearance of multipolar mitosis and lagging chromosomes at the metaphase plate. These effects correlate with prometaphase arrest and induction of caspase-dependent apoptosis or appearance of cells in a multinucleated interphase-like state unrelated to classical apoptosis pathways. Taken together, these results indicate that PM060184 represents a new tubulin binding agent with promising potential as an anticancer agent.

1. Introduction

Microtubules are major cytoskeletal components of eukaryotic cells that are involved in multiple cellular processes. Dynamic instability of microtubules determines the proper attachment of chromosomes to the spindle and their complex movements including their alignment at metaphase and separation at anaphase during mitosis [1–3]. Microtubules are also important in non-dividing cells were they regulate intracellular trafficking, vesicle transport, cell shape, cell movements and cell migration [4]. As microtubules are essential for many cell functions, they have been exploited as intracellular drug targets [5]. Tubulin-binding agents (TBA) are historically represented in the clinic by vinca alkaloids and taxanes [5]. These agents induce a sustained mitotic arrest at the metaphase/anaphase transition and subsequent apoptotic cell death [3,6]. Recently, cabazitaxel (a taxane), ixabepilone (an epothilone) and eribulin (an homohalicondrin derivative) were introduced in the clinic for the treatment of prostate and breast cancer. Moreover, brentuximab vedotin, an antibody–drug conjugate linking the chimeric monoclonal antibody brentuximab to monomethyl auristatin E (a marine-derived TBA) is used to treat anaplastic large cell lymphoma and Hodgkin lymphoma [7,8]. Similarly, trastuzumab emtansine, an antibody–drug conjugate incorporating

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http://dx.doi.org/10.1016/j.bcp.2014.01.026

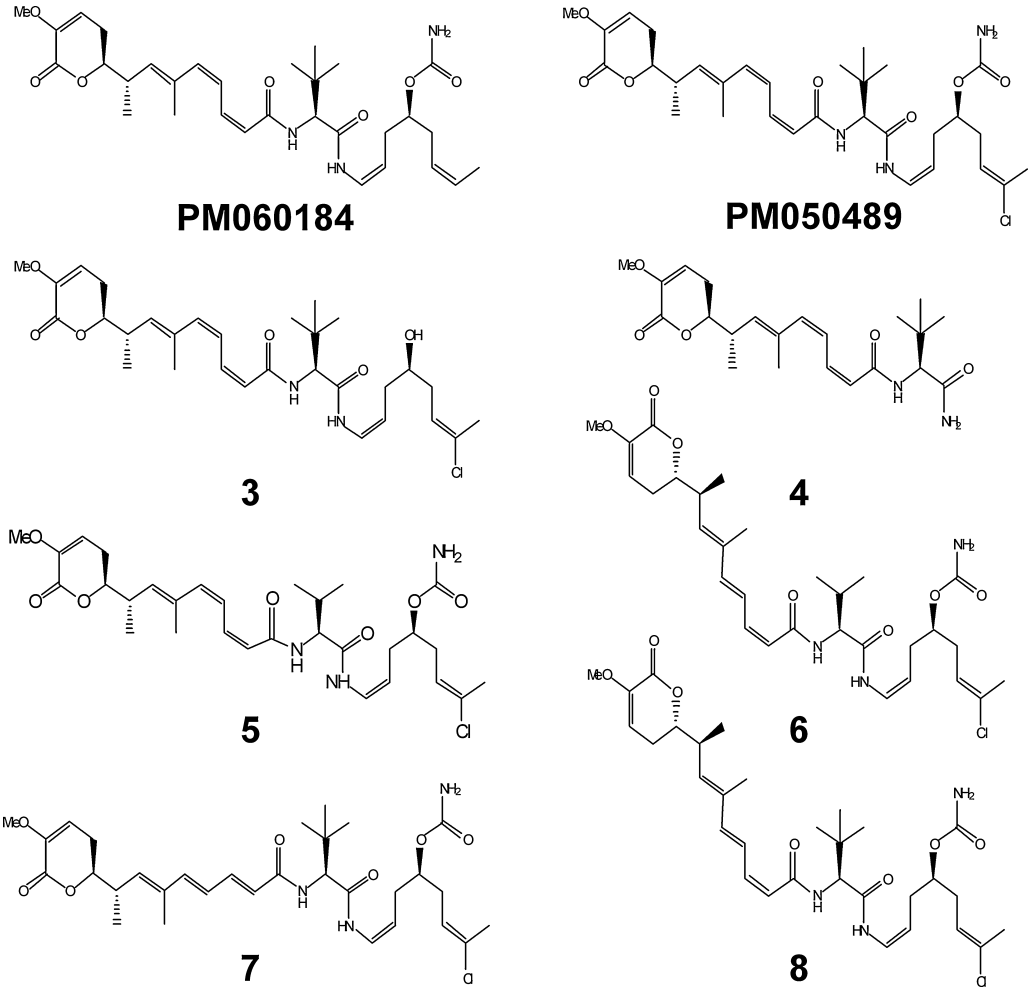

Fig. 1. Chemical structure of the compounds used in the study.

trastuzumab and the microtubule-inhibitory agent DM1, has been recently approved for the treatment of patients with HER2-positive advanced breast cancer [9,10]. Altogether, these data confirm that microtubules continue to be a valid target for cancer chemotherapy [11–13].

PM060184 (Fig. 1) belongs to a new family of TBA originally isolated from the marine sponge *Lithoplocamia lithistoides*, which are potent interfacial microtubule inhibitors with a mechanism of high-affinity binding to tubulin at a new site that is distinct from vinblastine [14,15]. PM060184 is currently produced by total synthesis and is under evaluation as a single agent in phase I clinical studies in patients with advanced cancer diseases [16,17]. In the present study, its *in vivo* antitumor activity was evaluated in a panel of different tumor xenograft models including P-glycoprotein (P-gp) overexpressors. In an attempt to shed light into the mechanism responsible of the observed antitumor activity, we have studied the effects of PM060184 in tumor cells including microtubule network structure and dynamics, centrosome modifications and type of cell death induced by this compound.

2. Materials and methods

2.1. Reagents

PM060184 and its analogs were isolated and/or synthesized at PharmaMar. Paclitaxel, vinblastine, vinorelbine, propidium iodide, Z-Vad-fmk, Hoechst 33258, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), rabbit polyclonal anti-γ-tubulin (T5192), mouse monoclonal anti-α-tubulin (T5168), mouse monoclonal anti-α-tubulin FITC conjugated (F2168) and anti-actin (A50–60) were obtained from Sigma (St Louis, MO, USA). Alexa 488-conjugated goat anti-rabbit IgG secondary antibody (A11078) and Alexa 594-conjugated goat anti-mouse IgG secondary antibody (A11032) were obtained from Molecular Probes (Life Technologies, Carlsbad, CA, USA). Primary rabbit polyclonal anti-PARP (sc-7150) and secondary peroxidase-conjugated anti-mouse or anti-rabbit IgGs were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Primary anti-phospho-Bcl-2 (2871) was purchased from Cell Signalling Technology (Danvers, MA, USA).

2.2. Animal studies

All animal protocols were reviewed and approved according to regional Institutional Animal Care and Use Committees. Female athymic *nu/nu* mice (Harlan Laboratories Inc., Italy) were housed in individually ventilated cages on a 12-h light–dark cycle at 21–23 °C and 40–60% humidity. Design, randomization and monitoring of experiments were performed using NewLab Software v2.25.06.00 (NewLab Oncology, Vandoeuvre-Lès-Nancy, France). Mice were subcutaneously implanted with MDA-MB-231 (breast), HCT-116 (colon), HGC-27 (gastric), H-460 (NSCLC), 22RV1 (prostate) and Caki-1 (renal) tumor cell lines. Mice bearing tumors (*ca.* 200 mm^3) were randomly allocated into experimental groups