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New imidazoquinoxaline derivatives: Synthesis, biological evaluation on melanoma, effect on tubulin polymerization and structure–activity relationships

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ABSTRACT

Microtubules are considered as important targets of anticancer therapy. **EAPB0503** and its structural imidazo[1,2-*a*]quinoxaline derivatives are major microtubule-interfering agents with potent anticancer activity. In this study, the synthesis of several new derivatives of **EAPB0503** is described, and the anticancer efficacy of 13 novel derivatives on A375 human melanoma cell line is reported. All new compounds show significant antiproliferative activity with IC_{50} in the range of 0.077–122 μM against human melanoma cell line (A375). Direct inhibition of tubulin polymerization assay *in vitro* is also assessed. Results show that compounds **6b**, **6e**, **6g**, and **EAPB0503** highly inhibit tubulin polymerization with percentages of inhibition of 99%, 98%, 90%, and 84% respectively. Structure–activity relationship studies within the series are also discussed in line with molecular docking studies into the colchicine-binding site of tubulin.

1. Introduction

Microtubules are the key components of the cytoskeleton of eukaryotic cells and have an important role in various cellular functions such as mitosis, exocytosis, and maintenance of cellular morphology, active transport, cell shape and polarization.[1,2] They play a critical role in cell division by their involvement in the movement and attachment of the chromosomes during various stages of mitosis. Therefore microtubule dynamics is an important target for the development of anti-cancer agents.[3–5] Microtubules are composed of α/β tubulin heterodimers, always in a state of equilibrium.[3,4] Microtubule targeting agents (MTA), drugs that interfere with microtubule dynamic stability, are widely employed in the clinic to treat a variety of cancers or are exploited as probes to gain insights into microtubule structure and function.[4–10] MTAs are antimitotic agents which perturb not only mitosis but also arrest cells during interphase. MTAs are known to interact with tubulin through at least four binding sites: the laulimalide, taxane/epothilone, vinca alkaloid, and colchicine sites.[1,11] Colchicine binds to the intradimeric α–β interface of tubulin heterodimers contiguous to the GTP-binding domain of the α-tubulin subunit[12–15] and the tubulin-colchicine complex prevents further polymerization of the microtubule. Such complex brings about a conformational change which blocks the tubulin dimers from further addition and prevents the growth of the microtubule by producing potent destabilization effects of microtubules, resulting in the subsequent shutdown of existing tumor vasculature.[1,16]

Efforts have been increasing for new compounds exhibiting inhibition of tubulin assembly and disassembly in order to induce anti-proliferative activities and treat a wide variety of malignancies.[17–22] The alteration of microtubule dynamics prevents cells division and apoptosis of different human cancer lines including Multi Drug Resistant (MDR) cancer cell lines.[23–25]

Imidazoquinoxalines derivatives have been designed by our laboratory since 2004 using different strategies of synthesis.

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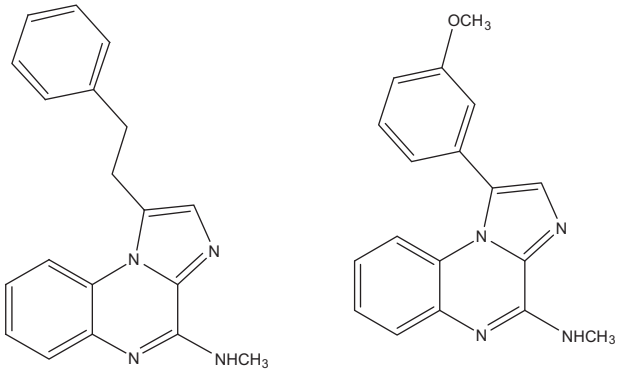


EAPB0203 EAPB0503

Figure 1. Chemical structure of lead compounds.

Among these derivatives, **EAPB0203** and **EAPB0503** (Fig. 1) have shown potent antitumor properties in vitro and in vivo against melanoma and T-cell lymphomas.[26–29] Preliminary studies have shown antitubulin activity for those two derivatives.[30]

Previous structure–activity relationship (SAR) studies with imidazoquinoxalines analogs were reported.[26,27] In continuation of previous work and in order to better study the possible correlation between the antiproliferative effect of our derivatives on human melanoma cell line and microtubule disruption, a new series of imidazoquinoxalines derivatives has been designed. Their biological activities were determined on the human melanoma cancer cell line A375 and possible correlations with their effect on tubulin polymerization were determined. Complementary molecular modeling studies on the colchicine binding domains of tubulin contribute to give new insights on structure activity relationships and possible development of the series.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of EAPB0503 derivatives

EAPB0503 and its derivatives have attracted our attention since 2004, due to their promising high potential activity against different types of cancer. They have been synthesized using a new strategy detailed previously.[26,27] Based on these encouraging first data on anticancer activity, new imidazoquinoxaline derivatives were designed. They have been obtained using the same strategy of synthesis. The advantage of this new synthetic method remains in the fact that all considered imidazoquinoxalines can be obtained from the same and common intermediate, the compound **1** (Scheme 1). The substitution for diversity is then performed at the last steps of the synthesis. The use of microwave assistance allows to dramatically optimize the time of reactions, purity of the intermediates, and global yields. It is also to be noted that the synthesis of the parent compound **1** can also be optimized using microwave assistance by treating the 4,5-lactame derivative precursor with phosphorus oxychloride and *N*,*N*-diethylaniline in a sealed vial using a Biotage® initiator microwave synthesizer. Nevertheless, the crude compound obtained still requires further purification on silica gel column chromatography for the next steps of the synthetic scheme.

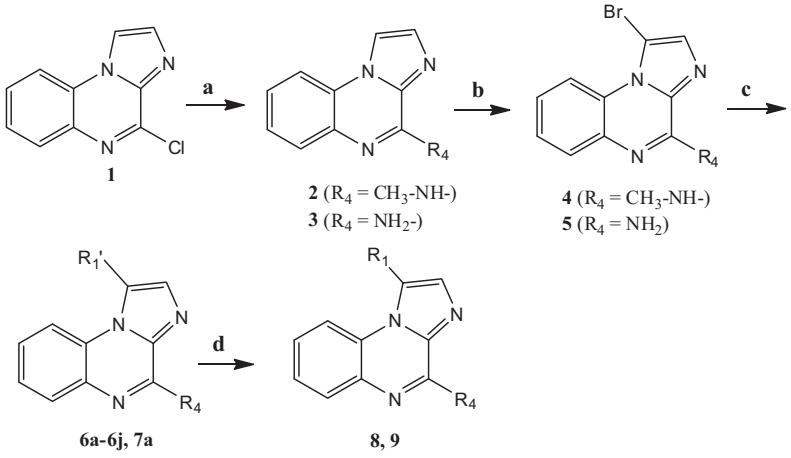
First diversity on position 4 is obtained by substitution of the chlorine on compound **1** by the appropriate amine under microwave heating to give compounds **2** and **3**. The bromination on position 1 of compounds **2** and **3** by *N*-bromosuccinimide under microwave conditions leads to compound **4** and **5**, respectively. In comparison to the initial synthetic procedure,[26–28] steps *a* and *b* (Scheme 1) as well as the step leading to the parent compound **1**, have been optimized using microwave irradiation instead of conventional heating.

A second step for diversity is obtained using different arylboronic acids in Suzuki-cross coupling reactions. As expected, the palladium catalyzed substitution selectively occurs on the bromo position to obtain compounds **6a** to **6j**, and **7a** under microwave assistance (140 °C, 20 min) with good to acceptable yields. The phenol derivatives **8** and **9** can be obtained by a treatment of the methoxy compounds **EAPB0503** and **7a** with iodocyclohexane under conventional heating. Compounds **2** to **4**, **EAPB0503**, **6a** and **6b** were described with their analytical data in previous studies.[26–28]

2.2. Biological evaluation

2.2.1. In vitro cell growth inhibition

All compounds were tested for their cytotoxic effect on human melanoma cell line (A375), our in vitro model used previously for screening,[26–28] using **EAPB0503** as reference, which showed previously an important cytotoxic activity comparing to fotemustine, clinically used for human melanoma treatment.[26–28] The screening results reported in Table 1 demonstrate that all our new



Scheme 1. Synthesis of **EAPB0503** derivatives. (a) EtOH, NH_2CH_3 in ethanol or NH_4OH in water, MW (180 °C, 20 min); (b) NBS, $CHCl_3$, reflux 1 h 30 min; (c) Suzuki-cross coupling (R_1′-$B(OH)_2$), $Pd(PPh_3)_4$, Na_2CO_3, DME, MW (140 °C, 20 min); (d) iodocyclohexane, DMF, reflux 16 h.