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Original article

Pyrimido[4,5-*c*]quinolin-1(2*H*)-ones as a novel class of antimitotic agents: Synthesis and in vitro cytotoxic activity

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Abstract

Several 2-amino-pyrimido[4,5-*c*]quinolin-1(2*H*)-ones variously substituted at positions 3, 5, and 9 were prepared from their corresponding lactones. The target compounds were investigated for in vitro cytotoxic activity against a panel of human cancer cell lines, namely, lung fibrosarcoma HT-1080, colon adenocarcinoma HT-29, and breast carcinoma MDA-MB-231. Analysis of data revealed that the presence of chloro at position 9 has a major positive impact on cytotoxic activity. Additional halogen substitution at the *para* position of the 3-phenyl group further enhances activity. Furthermore, compound (**25**) was found to dose-dependently inhibit tubulin polymerization. In accordance, flow cytometric analysis of the most potent compounds (**23**–**26**) indicated that the tested compounds induce cell cycle arrest in the G_2/M phase. The obtained results introduce the rarely described pyrimido[4,5-*c*]quinolin-1(2*H*)-one ring system as a new scaffold for promising antimitotic agents.

Keywords: Cytotoxic agents; Antimitotic; Pyrimido[4,5-*c*]quinolin-1(2*H*)-ones

1. Introduction

Recent revolutionary advances in the field of molecular biology and cancer cell biology have created new targets for antitumor compounds. Microtubules are hollow cylindrical protein polymers composed of α- and β-tubulin heterodimers. The tubulin heterodimers assemble to form the protofilaments which in turn associate longitudinally to form a microtubule as a long tube of 25 nm diameter [1]. During cell division, the microtubules are arranged in a unique manner to form the mitotic spindle which is the key cellular machinery driving mitosis in the metaphase where the replicated chromosomes are congressed to the equator, and the anaphase where chromosomes are segregated towards the spindle poles to generate two new daughter cells [2]. To perform this highly dynamic function, microtubules are required to be highly dynamic. In other words, microtubules should have the ability to lengthen and shorten through a reversible GTP-mediated process of association and dissociation of the α/β-tubulin heterodimers at both ends [3]. Microtubule-targeting drugs alter the dynamic behavior of microtubules through binding to tubulin thereby blocking mitotic cell progression and subsequently leading to apoptotic cell death [4–6]. Evidences indicate that even minor alteration of microtubule dynamics can have major consequences on cell cycle progression at mitosis [7–9]. Noteworthy, it has been postulated that mitosis can be blocked by abnormally rapid microtubule dynamics as well as by inhibited dynamics [10]. Two distinct categories of microtubule-targeting drugs are currently identified: agents that inhibit tubulin polymerization or ''microtubule-destabilizing agents'' such as the vinca alkaloids and colchicines, and agents that promote tubulin polymerization or ''microtubule-stabilizing agents'' such as the taxanes exemplified by paclitaxel and docetaxil [11]. For these reasons, tubulin became an attractive

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target in anticancer drug development. However, development of resistance and neurological, hematological, and other side effects significantly detracts from the therapeutic effectiveness of the currently available agents [12–14]. These findings sparked a worldwide search for new classes of drugs acting by the same mechanism yet enjoying better pharmacological profile.

In the past two decades, there has been a growing interest in 2-phenyl-4-quinolones (A), the aza bioisosteric analogues of flavones (B), as potent cytotoxic antimitotic agents interacting with microtubules [15–21]. Likewise, natural flavonoids have been reported to display potent cytotoxic activity through inhibition of tubulin polymerization [22,23]. Meanwhile, 2-phenyl-4-anilinoquinolines (C) and related condensed heterocyclic compounds were extensively reported to have appreciable cytotoxic activities [24–29]. It was generally concluded that not only the coplanar polycyclic core but also peripheral substituents have considerable impact on cytotoxic activity. In the same context, we report herein the synthesis and the in vitro cytotoxic activity of pyrimidio[4,5-*c*]quinolin-1(2*H*)-ones (D) as a new class of antimitotic agents (Chart 1). The mechanism of action of the new agents was explored through cell cycle analysis performed by a flow cytometric assay and a tubulin polymerization assay.

2. Chemistry

Synthetic route to the target pyrimido[4,5-*c*]quinolin-1(2*H*)-ones (**16–26**) is illustrated in Scheme 1. Phenacylamine hydrochlorides (**1** and **2**) were prepared according to a standard method, as reported previously [30]. The starting 3-amino-2-arylquinoline-4-carboxylic acids (**3–6**) were synthesized following a modified Pfitzinger procedure by reacting isatin or 5-chloroisatin with 4-chloro- or 4-bromophenacylamine hydrochlorides (**1** and **2**) under basic conditions [30,31]. Subsequently, the acids were cyclized with acetic anhydride under reflux conditions to afford the key lactones 3-methyl-5-aryl-1*H*-[1,3]oxazino[4,5-*c*]quinolin-1-ones (**7** and **8**) in good yields. Alternatively, reacting the starting acids (**3–6**) with benzoyl chloride or 4-chlorobenzoyl chloride at room temperature followed by cyclization of the resulting intermediate using acetic anhydride gave the lactones (**9–15**) in moderate overall yields. Finally, the requisite pyrimido[4,5-*c*]quinolin-1(2*H*)-ones (**16–26**) were obtained via hydrazinolysis of the intermediate lactones (**7–15**) through heating with hydrazine hydrate in 2-ethoxyethanol as a high boiling solvent.

Structure of the target pyrimido[4,5-*c*]quinolin-1(2*H*)-ones (**16–26**) as well as the intermediate lactones (**7–15**) was confirmed by means of IR, ^{1}H NMR, mass spectral data and microanalysis. IR spectra of (**7–15**) showed a characteristic lactone carbonyl stretching frequency around 1765 cm^{-1}. Mass spectra revealed the appearance of isotopic peaks owing to the presence of halogens. Unfortunately, these intermediate lactones could not be characterized by ^{1}H NMR spectrometry due to lack of solubility in the available ^{1}H NMR solvents such as $CDCl_3$, DMSO-d_6, CD_3OD, CD_3CD_2OD and CD_3COCD_3. In the ^{1}H NMR spectra of the target compounds (**16–26**), a singlet peak in the region δ 6.0–6.3 integrating for two protons was characteristic of the NH_2 group. The methyl protons of compounds (**16**), (**17**) and (**22**) appeared as a sharp singlet at δ 2.7. Aromatic protons appeared at their expected chemical shifts. All the new compounds were microanalyzed satisfactorily for C, H, N.

3. Results and discussion

The widely accepted MTT assay was used for the in vitro cytotoxic evaluation of the target compounds (**16–26**) against

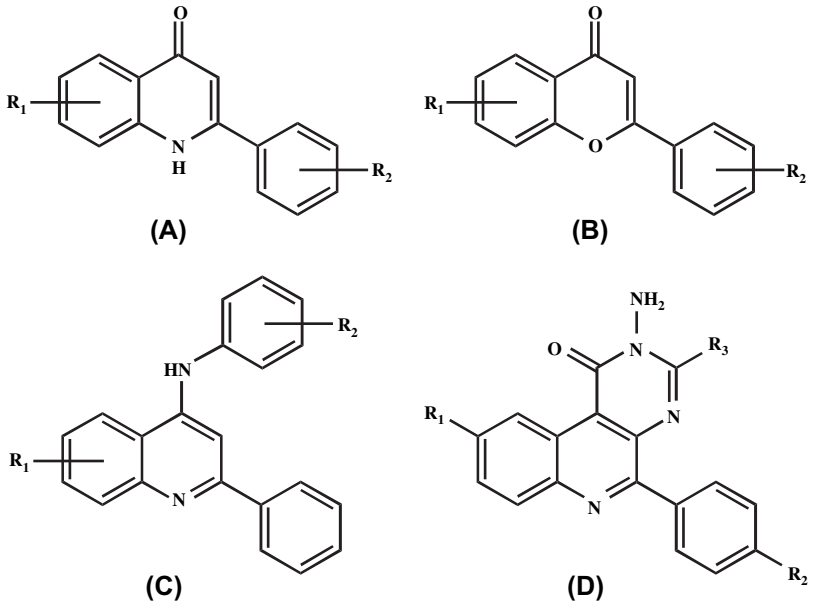

Chart 1.