European Journal of Medicinal Chemistry 143 (2018) 8–20

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis and biological evaluation of *N*-substituted 3-oxo-1,2,3,4-tetrahydro-quinoxaline-6-carboxylic acid derivatives as tubulin polymerization inhibitors

Jianguo Qi, Haiyang Dong, Jing Huang, Shufeng Zhang, Linqiang Niu, Yahong Zhang*, Jianhong Wang**

Key Laboratory of Natural Medicine and Immuno-Engineering of Henan Province, Henan University Jinming Campus, Kaifeng, 475004, Henan, China

ARTICLE INFO

Article history:
Received 2 October 2016
Received in revised form
5 August 2017
Accepted 6 August 2017
Available online 7 August 2017

Keywords:
Antiproliferative
Tubulin polymerization inhibitors
Cell cycle analysis
Cell apoptosis
3-Oxo-1,2,3,4-tetrahydro-quinoxaline-6-carboxylic acid derivatives

ABSTRACT

A series of novel *N*-substituted 3-oxo-1,2,3,4-tetrahydro-quinoxaline-6-carboxy- lic acid derivatives were synthesized and evaluated for their biological activities. Among all synthesized target compounds, **13d** exhibited the most potent antiproliferative activity against HeLa, SMMC-7721, K562 cell line (IC_{50} = 0.126 μM, 0.071 μM, 0.164 μM, respectively). Furthermore, compound **13d** inhibited tubulin polymerization (IC_{50} = 3.97 μM), arrested cell cycle at the G2/M phase and induced apoptosis. The binding mode at the colchicine binding site was also probed. These studies provided a new molecular scaffold for the further development of antitumor agents that target tubulin.

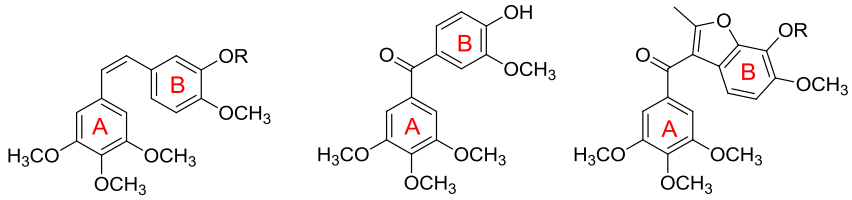
1. Introduction

Microtubules, which are dynamic polymers of αβ-tubulin, play an essential role in mitosis, forming the dynamic spindle apparatus. Disruption of microtubule dynamics prevents microtubule function and ultimately leads to cell death. This made microtubules an important target for cancer chemotherapy. Microtubule-targeting agents are divided into two groups, microtubule-stabilizing agents (paclitaxel, laulimalide etc) and tubulin polymerization inhibitors (vinca alkaloids, colchicine etc). These agents interact with tubulin through three major binding sites, vinca alkaloid-, taxane- and colchicine-binding sites [1–3]. Recently, diverse small molecules that act at the colchicine site on tubulin have come under intensive investigation. These compounds not only display potent cytotoxicity against a wide variety of human cancer cell lines but also show selective toxicity toward tumor endothelial cells required for the growth of the cancer. Thus they represent a new class of vascular disrupting agents which cause a significant shutdown of the blood vessels of tumors, leading to cancer cell death via necrosis and apoptosis. Moreover, they show promising ability to overcome multidrug resistance mediated by P-glycoprotein [4–14]. Therefore, this type of tubulin inhibitor might provide a new opportunity for cancer therapy.

Currently, some drug candidates targeting at the colchicine site are in clinical development. CA4-P (**1a**, Fig. 1), the phosphate derivative prodrug of combretastatin A-4 (CA-4, **1b**, Fig. 1), has entered clinical trials either alone or in combination with other chemotherapeutic agents [15,16]. However, the *cis*-stilbene of CA-4 is prone to isomerisation into its inactive *trans*-form during storage and administration. To avoid the stability problems of CA-4, many conformationally restricted modification have been used [17–23]. Phenstatin (**2**, Fig. 1) which is the CA-4 analogue with the double bond of CA-4 being replaced by a carbonyl group showed strong cytotoxicity and antitubulin activity similar to CA-4, but it is more stable compared with CA-4 [24]. BNC-105P (**3a**, Fig. 1), developed by Bionomics (Australia), is a phosphorylated prodrug of BNC-105 (**3b**, Fig. 1) and it entered clinical trials in combination with everolimus for progressive metastatic clear cell renal cell carcinoma. It is the phenstatin analogue with the benzene ring (ring B) of phenstatin being replaced by coumarone [25]. Based on BNC-105

* Corresponding author.
** Corresponding author.
E-mail addresses: zhangyahong_131@163.com (Y. Zhang), jhworg@126.com (J. Wang).

https://doi.org/10.1016/j.ejmech.2017.08.018
0223-5234/



1a (CA-4P) R = $P(O)O_2Na_2$
1b (CA-4) R = H
2 (Phenstatin)
3a (BCN-105) R = $P(O)O_2Na_2$
3b (BCN-105P) R = H

Fig. 1. CA-4 and its analogues targeting at the colchicine site of tubulin.

and other modification of CA-4 and phenstatin, we conclude that ring B could be replaced by other heterocycles, such as chromane, indole etc [26–41].

Quinoxalinone structure has good physico-chemical properties and drug-like properties. Many bioactive compounds contain this moiety [42,43]. The current study was undertaken to investigate the in vitro antitumor activity of a novel modification of phenstatin. The replacement of one phenyl ring with 3-oxo-1,2,3,4-tetrahydro-quinoxaline-6-carboxylic acid and its derivatives were pursued. Herein we report the synthesis and biological evaluation of a series of *N*-substituted 3-oxo-1,2,3,4- tetrahydro-quinoxaline-6-carboxylic acid derivatives. Compound **13d** was identified with IC_{50} values ranging from 0.071 to 0.164 μM against the three cancer cell lines. The effects on tubulin polymerization, cell cycle and apoptosis were also evaluated.

2. Results and discussion

2.1. Chemistry

Scheme 1 depicts the synthetic pathways used for the preparation of quinoxalinone derivatives. Starting from the commercial compound 4-amino-3- nitrobenzoic acid (**4**), the reaction with thionyl chloride in methanol and properly substituted amines in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) in CH_2Cl_2 provided the compounds **5a-5e** (71.8%–89.2%). These latter compounds reacted with ethyl bromoacetate in the presence of Cs_2CO_3 to afford compounds **6a-6e** (44.3%–58.3%). The key intermediates **7a-7e** (68.8%–82.4%) were synthesized via a "one pot" reduction of the nitro group using hydrogen in the presence of palladium on charcoal and cyclization reaction of **6a-6e** in methanol. Compounds **8a-8c** (42.3%–67.8%) were prepared by nucleophilic substitution of substituted benzyl chlorides with intermediates **7a-7e** using K_2CO_3 as a base. The subsequent hydrolysis of compound **8c** using LiOH aqueous solution furnished the desired compound **9** (72.6%). Compound **9** was converted into its acid chloride with oxalyl chloride in toluene. Compounds **10a** (73.2%) and **10b** (62.4%) were prepared by condensation of the acid chloride mentioned above with properly substituted amines in the presence of K_2CO_3 in tetrahydrofuran (THF). Compounds **13a-13i** (46.7%–65.3%) were prepared by nucleophilic addition of intermediates **7a-7e** with substitued benzoyl chlorides in the presence of K_2CO_3 in THF. The benzoyl chlorides **12a-12e** were gained by treatment of substituted benzoic acids with thionyl chloride.

2.2. Biological evaluation

2.2.1. In vitro antiproliferative activity

The in vitro antiproliferative activities of the synthesized target compounds were evaluated against HeLa (human epithelial cervical cancer), SMMC-7721(human hepatoma cancer), and K562 (leukemia) cell lines using MTT assay. Doxorubicin and CA-4 were chosen as reference drugs. The results are summarized in Table 1. The antiproliferative activities of the compounds were expressed as the concentration of compounds required for 50% inhibition of cell growth (IC_{50}). IC_{50} values were calculated from at least five different concentrations of test compounds.

As shown in Table 1, most of the synthesized compounds inhibited the growth of the three cancer cells with IC_{50} values under 50 μM. The comparison of IC_{50} values of **8a** − **8c** demonstrated that **8c** which had 3,4,5-trimethoxybenzyl substitution on the nitrogen atom of methyl 3-oxo-1,2,3,4-tetrahydroquinoxaline-6-carboxylate had better activity than **8a** (4-methoxybenzyl) and **8b** (3,4-dimethoxybenzyl). The activity decreased by an order of magnitude when the methyl carboxylate of **8c** was hydrolyzed to carboxylic acid (**9**). Compound **10a** or **10b** with *N*-butyl or *N*-cyclohexyl quinoxalinone-6-carboxamide substitued for quinoxalinone-6-carboxylate of compound **8c** showed comparable activity with compound **9**.

Comparison of compounds **13b**–**13d** with compounds **8a** − **8c** revealed that the replacement of benzyl group with benzoyl group led to a remarkable improvement in antiproliferative activity of the corresponding compounds. The antiproliferative activity of compounds **13b**–**13d** with different substituents on the benzene increased in the following order: **13d** (3,4,5-trimethoxy) > **13c** (3,4-dimethoxy) > **13b** (4-methoxy). Compounds **13d** and **13c** were the two most potent compounds among all the synthesized target compounds. Compound **13d** exhibited better activity than doxorubicin, but less than CA-4. Compound **13a** with *para*-fluoro substituent on the phenyl ring showed comparable activity with **13b** (4-methoxy). Lengthening the benzoyl moiety of **13d** to phenylacetyl group (**13e**) caused a significant reduction in antiproliferative activity in all cell lines. Replacement of methyl carboxylate on the quinoxalinone with amide (**13f-13l**) led to weak activity.

2.2.2. In vitro inhibition of tubulin polymerization

Three compounds, including **8c**, **13c** and **13d**, were selected to evaluate for their ability to inhibit tubulin polymerization and they were employed at 20 μM in the assay. CA-4 (2.5 μM) and paclitaxel (3 μM) were evaluated as reference compounds. Compound **13d** was employed at four different concentrations in order to determine the IC_{50}. In the assay, the IC_{50} was defined as the concentration of compound that inhibited 50% the extent of assembly of 10 μM tubulin after 40 min incubation at 37 °C. As shown in Fig. 2A, **8c**, **13c** and **13d** resulted in various degrees of inhibition of tubulin polymerization compared with control (0.1% DMSO). Compound **13d** inhibited tubulin polymerization with an IC_{50} value of 3.97 μM.