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Research paper

Synthesis and biological evaluation of novel non-racemic indole-containing allocolchicinoids

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ABSTRACT

Two novel indole-containing allocolchicinoids were prepared from naturally occurring colchicine exploiting the Curtius rearrangement and tandem Sonogashira coupling/Pd-catalyzed cyclization as the key transformations. Their cytotoxic properties, apoptosis-inducing activity, tubulin assembly inhibition and short-time cytotoxic effects were investigated. Compound **7** demonstrated the most pronounced anti-cancer activity: IC_{50} < 1 nM, cell cycle arrest in the G2/M phase, 25% apoptosis induction, as well as lower destructive short-time effects on HT-29 cell line in comparison with colchicine. Docking studies for prepared indole-derived allocolchicine analogues were carried out.

1. Introduction

Tubulin proteins play an essential role in many cellular functions of eukaryotic cells such as cell proliferation, the development of cell shape, cell motility, and cell signaling [1]. Colchicine (**1**), isolated from *Colchicinum autumnale*, was the first tubulin-binding agent ever discovered [2]. For a long time, colchicine was considered as a perspective antitumor agent. However, its significant general toxicity even in therapeutic doses prevented its use in cancer therapy [3]. On the other hand, the tubulin-destabilizing properties of colchicine allow its use in clinical practice for the treatment of familial Mediterranean fever, Behcet's disease, as well as several types of microcrystalline arthritis [4]. Recently, colchicine was suggested as a drug for the treatment of cardiovascular diseases, such as acute pericarditis, atrial fibrillation caused by inflammation, and ischemic diseases [5]. These properties, obviously, are associated with the ability of colchicine to accumulate in the immune system cells, leading to the suppression of inflammatory reactions. Potentially, colchicine and its analogues may be useful in the treatment of autoimmune, allergic and neurodegenerative diseases, and in therapy of chronic infections [6,7]. Therefore, the search for less toxic colchicine-binding site inhibitors [2c] with improved bioavailability and tissue distribution might lead to the elaboration of novel therapeutic agents, useful in the treatment of inflammatory, cardiovascular, autoimmune and oncological diseases.

Our group has recently synthesized a series of heterocyclic allocolchicine analogues of colchicine, containing indole (**2-4**) [8] or benzofurane (**5** and **6**) [9] fragments replacing the C-ring in the parent compound (Fig. 1).

These molecules have demonstrated higher activity as tubulin

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polymerization and cell proliferation inhibitors compared to colchicine, while their general cytotoxicity was relatively low. Thus, furano-allocolchicinoids **5** inhibit tumor growth in mice without displaying symptoms of acute toxicity such as weight loss or neurological disorders, typical for colchicine poisoning.

2. Results and discussion

2.1. Chemistry

Racemic pyrrolo-allocolchicinoids **2-4** were prepared previously in 11 steps starting from 3,4,5-trimethoxyphenylpropionic acid [8]. Herein, we report a conceptionally different ten-steps semi-synthetic approach (Scheme 1) towards non-racemic pyrrolo-allocolchicine **7** (Fig. 1) starting from naturally occurring (-)-(a*R*, 7*S*)-colchicine **1**. Previously, we observed that the presence of a hydroxymethyl substituent either at C-7 of ring B (compounds **2-4**) [8a] or on the heterocyclic fragment (compounds **5** and **6**) [9] significantly increases the anti-proliferative activity. Thus, a similar 2″-hydroxymethyl substitution pattern in the pyrrole fragment was chosen for these new synthetic targets.

The bromination of colchicine (**1**) using NBS in TFA/AcOH mixture afforded 4-bromocolchicine in almost quantitative yield. It was transformed into 4-bromoallocolchicinic acid **8** by applying the previously described base-induced ring contraction [10] (85% yield over two stages). Allocolchicinic acid **8** was subjected to the Curtius degradation yielding the corresponding aniline **9** in 45% yield. The followed α-iodination reaction (NIS/AcOH) and subsequent acylation gave allocolchicinoid **10** in 64% yield. Finally, the desired *N*-methylated pyrrolo-allocolchine **7** was prepared in 3 steps from the amide **10**. The TBDMS-protected indolyl-2-methanol **11** was obtained through tandem Sonogashira reaction/cyclization sequence in 51% yield [11], using O-protected propargyl alcohol. After the N-methylation of the indole nitrogen (NaH/MeI) and selective cleavage of the silyl group, compound **7** was isolated in 55% yield over two steps.

In order to determine the influence of substituents at the nitrogen atom on the biological activity, *N*-H pyrrolo-allocolchine **12** was also prepared, starting from amide **10** via two-step one-pot Sonogashira/Larock-type cyclization [11], using unprotected propargyl alcohol for the coupling stage. It has been demonstrated, that the presence of a halogen atom in the A-ring of colchicinoids slightly enhances their cell inhibitory activity [12], thus the presence of bromine in the final products was considered as positive.

2.2. Biology

2.2.1. Cytotoxicity

The cytotoxicity of the synthesized compounds towards human pancreatic adenocarcinomas COLO-357 and MiaPaCa-2, and human embryonic kidney cell line HEK293 was investigated *in vitro*. A tetrazolium-based assay was used to determine the drug concentration required to inhibit cell growth by 50% (IC_{50}) after incubation in the culture medium for 72 h. The calculated IC_{50} values are summarized in Table 1. Compound **12** was less active than colchicine. In contrast, *N*-methylated analog **7** inhibited cell proliferation already at subnanomolar concentrations, lower than of colchicine. We presume that the presence of alkyl on the *N*-1 of the pyrrolo-allocolchicine skeleton is essential for the antiproliferative activity of the prepared compounds.

2.2.2. Cell cycle analysis

The main mechanism of the colchicine action is the inhibition of tubulin assembly, which leads to the block of cell proliferative cycle and subsequent apoptosis. We found that both new compounds demonstrate colchicine-specific tubulin interaction (Figs. 2 and 3). When compared in the equal concentrations, the only difference found was the higher activity of compound **7** in cell cycle arrest (Fig. 2, Table 2) expressed by the increased number of cells in G2/M phase, and higher percentage of cells in the apoptotic fraction.

Similarly, a higher activity in tubulin assembly inhibition was found in cultures stained with anti-β-tubulin antibodies (Fig. 3a-d).

Tubulin assembly inhibition, resulting in the mitotic spindle block and G2/M cell cycle arrest, requires at least 20-24 h to proceed completely (see Fig. 2A-D). However, colchicine and its analogues bind to tubulin much faster. Incubation of cells with compound **7** for 3 h already re-localized β-tubulin (Fig. 3e-f). The cytotoxic effects of colchicine and its analogues *in vitro* are rather mild, and require 72 h to reach maximum 60-80% inhibition. Short-time cell incubation with colchicine derivatives demonstrated that

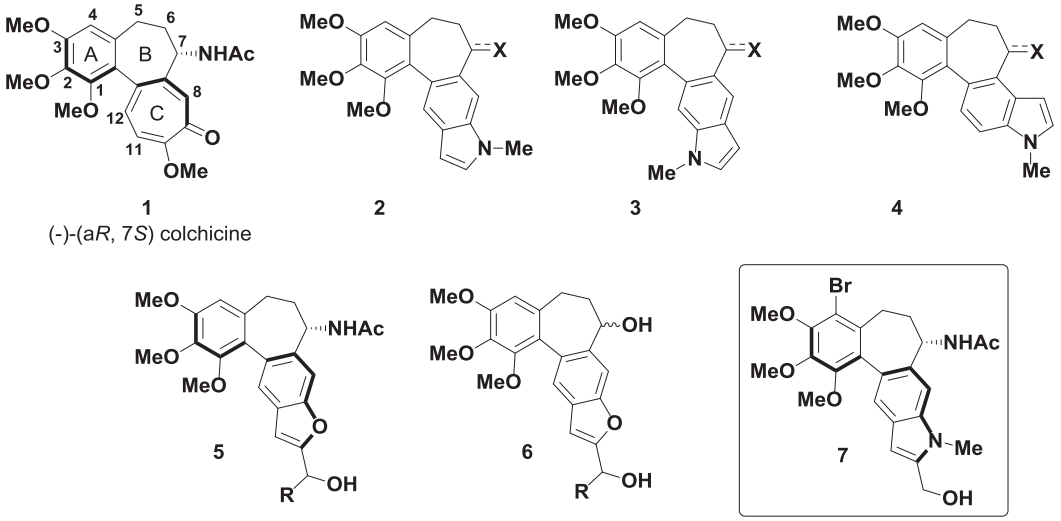

Fig. 1. Colchicine and its heterocyclic analogues.