Bioorganic Chemistry 64 (2016) 103–112

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

Synthesis, antiproliferative activity and molecular docking of Colchicine derivatives

Adam Huczyński [a,*], Urszula Majcher [a], Ewa Maj [b], Joanna Wietrzyk [b], Jan Janczak [c], Mahshad Moshari [d], Jack A. Tuszynski [d], Franz Bartl [e]

[a] Faculty of Chemistry, Adam Mickiewicz University, Umultowska 89b, 61-614 Poznań, Poland
[b] Ludwik Hierszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 12, 53-114 Wrocław, Poland
[c] Institute of Low Temperature and Structure Research, Polish Academy of Sciences, PO Box 1410, 50-950 Wrocław, Poland
[d] Department of Oncology, University of Alberta, Edmonton, Alberta T6G 1Z2, Canada
[e] Institut für Medizinische Physik und Biophysik Charité – Universitätsmedizin Berlin, Campus Charité Mitte, Charitéplatz 1, 10117 Berlin, Germany

ARTICLE INFO

Article history:
Received 17 November 2015
Revised 7 January 2016
Accepted 8 January 2016
Available online 12 January 2016

Keywords:
Antimitotic agent
Tubulin
Colchicine derivatives
Crystal structure
Cytotoxicity
Docking

ABSTRACT

In order to create more potent anticancer agents, a series of five structurally different derivatives of Colchicine have been synthesised. These compounds were characterised spectroscopically and structurally and their antiproliferative activity against four human tumour cell lines (HL-60, HL-60/vinc, LoVo, LoVo/DX) was evaluated. Additionally the activity of the studied compounds was calculated using computational methods involving molecular docking of the Colchicine derivatives to β-tubulin. The experimental and computational results are in very good agreement indicating that the antimitotic activity of Colchicine derivatives can be readily predicted using computational modeling methods.

1. Introduction

Colchicine (**1**), the major alkaloid isolated from *Colchicum autumnale* and *Gloriosa superba* is a well-known antimitotic agent, which has been shown to exhibit very high cytotoxic effects *in vivo* [1,2]. Microtubules, formed by polymerization of tubulin hetero-dimers, are essential components of the cytoskeleton in eukaryotic cells and are involved in many important cellular processes, including mitosis [3,4]. Colchicine binds at a unique binding site close to the interface between α- and β-tubulin but makes contact solely with β-tubulin. Colchicine binding results in the formation of a tubulin–Colchicine complex that prevents the formation of microtubules due to induced conformational change in the tubulin dimer making it incompetent for microtubule assembly. As a result the cells exposed to Colchicine exhibit apparent mitotic arrest during the cell cycle [5–7]. Colchicine has been shown to have very high anti-cancer (specifically anti-mitotic) activity *in vitro* but its use as an anti-cancer drug have not led to clinical applications because of its relatively high toxicity, although Colchicine is currently used in therapy [8,9], i.e. for gout [3,10,11], acute pericarditis [12,13] and Familial Mediterranean fever (FMF) [14,15]. Colchicine remains a molecule of great research interest since Colchicine site inhibitors (CSI) as a class have been most widely explored compared to the other binding sites of tubulin [16,5]. Colchicine is a compound whose structure is still being optimized, by designing new analogues Some derivatives of **1**, such as thiocolchicoside (Neoflax™, Muscoril™) show improved therapeutic properties and clinical significance as anti-inflammatory, analgesic and anti-cancer drugs [17,18].

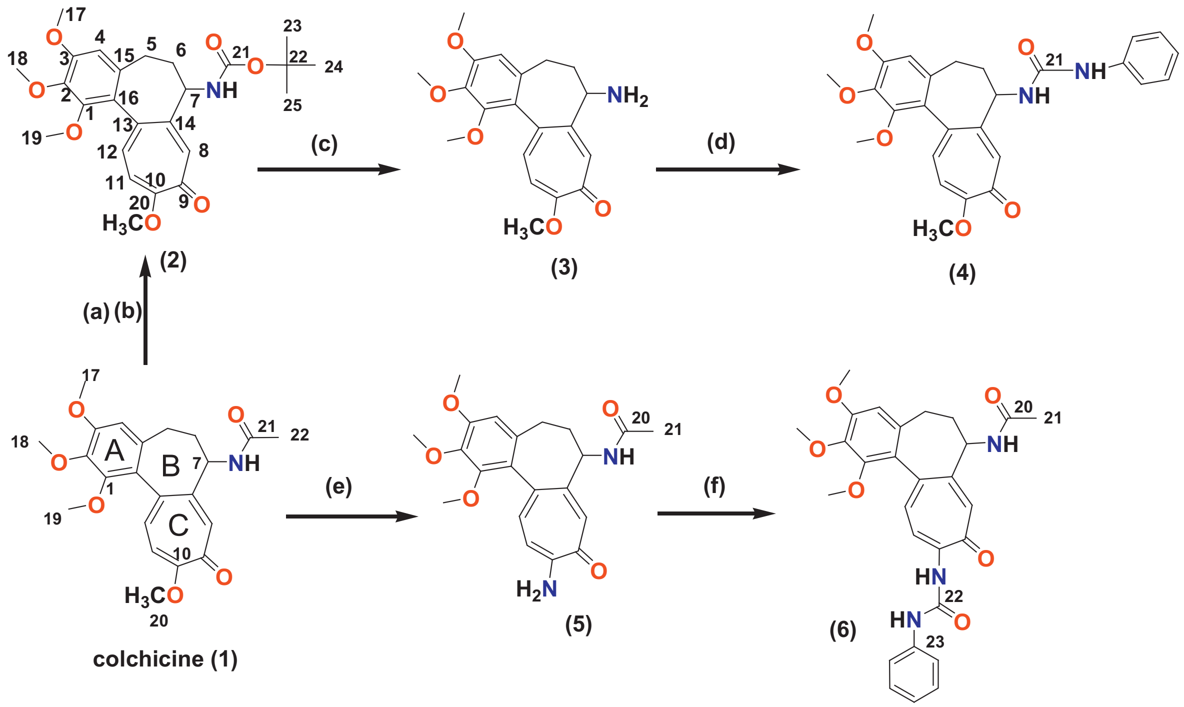
Therefore, currently much interest has focused on structural modifications of **1** in the hope of improving its anti-cancer activity. Numerous studies have shown previously that changing of the $-OCH_3$ group at the C(10) position of C-ring into an amine, as well as substitution of the acyl group at C(7) position of B-ring into several new derivatives may reduce the toxicity of **1** toward normal cell; thus, this kind of modifications may lead to improved anti-cancer agents [19–26].

The main purpose of our study was to design and synthesize (Scheme 1) a few derivatives of Colchicine showing considerable activity against human cancer cell lines.

---

* Corresponding author.
E-mail address: adhucz@amu.edu.pl (A. Huczyński).

http://dx.doi.org/10.1016/j.bioorg.2016.01.002



Scheme 1. Synthesis of Colchicine derivatives (**2–6**). *Reagents and conditions*: (a) Boc_2O, DMAP, triethylamine, CH_3CN, reflux; (b) MeONa, MeOH, RT; (c) Trifluoroacetic acid, DCM, reflux; (d) phenyl isocyanate, tetrahydrofuran, reflux; (e) $NH_3(aq)$, C_2H_5OH, reflux; (f) phenyl isocyanate, acetone, reflux.

Below, we report the synthesis, crystallographic and spectroscopic analysis of a series of structurally different derivatives of **1** obtained by the modification at C(10) and C(7) positions of **1** as well as evaluation of these novel compounds as cytotoxic, tubulin-targeting agents. The antiproliferative effect of five Colchicine derivatives (**2–6**) was tested *in vitro* using four cancer cell lines, i.e.: human promyelocytic leukemia (HL-60) and its vincristine-resistant subline (HL-60/vinc), human colon adenocarcinoma cell line (LoVo) and doxorubicin resistant subline (LoVo/DX), and one normal murine embryonic fibroblast cell line (BALB/3T3). To better understand the interactions between the Colchicine derivatives and tubulin, we investigated potential binding modes of all studied compounds (**1–6)** docked into the Colchicine binding site (CBS) of βI tubulin using Autodock4 program under flexible ligand and rigid receptor condition. A detailed discussion regarding the differences between the structures of the synthesized compounds and their ability to form complexes with Colchicine binding site (CBS) is provided herein.

2. Results and discussion

2.1. Chemistry

The synthesis routes to Colchicine derivatives **2–6** are outlined in Scheme 1. Compounds **2**, **3** and **5** were prepared according to the methods developed earlier [25,26]. Compounds **4** and **6,** containing *N*-phenyl urea substituent, were synthesized and characterized for the first time.

According to Bagnato's method [25] *N*-deacetylcolchicine (**3**) was synthesized in three steps as follows: Colchicine (**1**) reacted with Boc-anhydride (Boc_2O) using 4-dimethylaminopyridine (*DMAP*) forming *N*-Boc-colchicine (not isolated), followed by selective methanolysis of the acetate group giving *N*-Boc-deacetylcolchicine (**2**) and the subsequent reaction with *trifluoroacetic acid* (TFA) yielded the desired *N*-deacetylcolchicine (**3**). Isolated compound **2** was crystallized from acetonitrile producing crystals, which are analyzed by X-ray diffraction. A simple reaction between **3** and phenyl isocyanate in tetrahydrofuran at room temperature gives compound **4** with a 68% yield. Compound **5** was readily available from **1** by treatment with excess of aqueous ammonia solution in ethanol and heat under reflux for 12 h [26]. The reaction proceeds quantitatively. Compound **6** was obtained from **5** by a reaction with phenyl isocyanate in acetone at room temperature and then after chromatographic purification and crystallization from acetonitrile (final yield 55%). The crystals are suitable for X-ray analysis whose results are presented and discussed below. The structures of all products **2–6** were determined using the EI MS, ESI-MS, FT-IR, ^1H and ^{13}C NMR methods and are shown in Supplementary data (Figs. S1–S18) and discussed below. All the spectroscopic and mass spectrometry data presented in Supplementary data confirm the structure of the studied compounds. Additionally, the structures of compounds **2** and **6** are also determined strictly from X-ray analysis presented also in Supplementary data (Tables S1–S3, Figs. S19 and S20).

The structural characterization of the Colchicine derivatives is very important in order to understand their anticancer properties stemming from their interaction with tubulin as well as to enable structure–activity relationship analysis (SAR) and related investigation [5,27–41]. Therefore, we have characterized two Colchicine derivatives **2** and **6** that produce appropriate crystals suitable for the single crystal X-ray analysis. The single crystals of **2** and **6** were grown by crystallization in acetonitrile solution (Table S1, Fig. S19).

Compound **2** crystallizes in the *C*2 space group of the monoclinic system while compound **6** in the $P2_12_12_1$ space group of the orthorhombic system. Both space groups are chiral since the compounds contain an asymmetric carbon (C7) atom. The planar phenyl A and tropolone C rings in both Colchicine derivatives (**2** and **6**) are twisted around the C13–C16 bond with the torsion angle describing the twisting conformation C1–C16–C13–C12 of 57.17 (23)° and 52.06(26)° in **2** and **6**, respectively. The ring B in both compounds exhibits a similar puckering pattern and the extent of its non-planarity and adopts a conformation, which is close to