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STABILIZATION OF MICROTUBULES BY COMBRETASTATIN D DERIVATIVES

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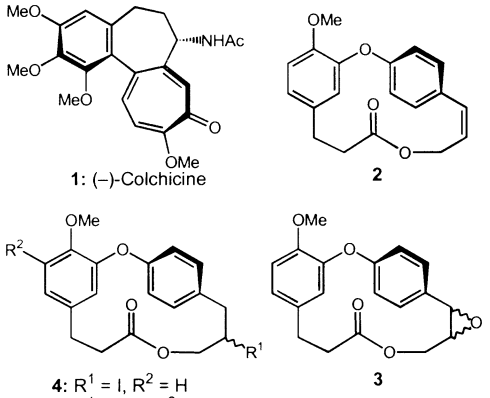
Abstract: The effect of five derivatives of Combretastatin D on tubulin polymerization was investigated. All of them were found to stabilize microtubules to various degrees. The derivatives bearing polar substituents were found to be the most active. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

Microtubules are important biopolymers that not only comprise the cytoskeleton of eukariotic cells but are also involved in diverse cellular processes such as cell division, locomotion, and intracellular transport. As a consequence tubulin polymerization/depolymerization is a popular target for new chemotherapeutic agents. Among the various classes of compounds that affect this bioproccess (Vinca alkaloids, taxoids, epothilones, podophylotoxins, etc.) several binding sites as well as different interaction modes have been discovered.[1,2]

Combretastatins of the D series are a new class of natural products showing significant cytotoxic activity.[3] These compounds posses two neighboring aromatic moieties in a more or less perpendicular spatial arrangement. This is a common structural feature of colchicine, the archetypal tubulin-binding antimitotic drug, as well as of phenyl-tropones, steganacins, podophylotoxins, combretastatin and combretastatins A. Notwithstanding the structural similarity of combretastatins D with the above mentioned compounds, the possibility of them having similar biological target has not been investigated so far due to their scarcity in nature.

Figure 1. (–)-Colchicine and Combretastatin D derivatives



1: (–)-Colchicine

2

3

4: R^1 = I, R^2 = H
5: R^1 = OH, R^2 = H
6: R^1 = OH, R^2 = Br

We have already reported[4] on an efficient total synthesis of this class of compounds developed in our laboratory that enabled us to prepare the naturally occurring compounds as well as several derivatives. We

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would like to present here our preliminary observations concerning the interaction of these compounds with tubulin.

Results and Discussion

The effect of some Combretastatin D derivatives[4] (**2–6**; Figure 1) on tubulin polymerization at different concentrations was determined by the filtration-colorimetric method developed by Bollag et al.[5] and the results are summarized in Table 1. We were pleased to find out that all tested compounds interfere with the polymerization of tubulin. However in contrast to colchicine and combretastatins A which inhibit tubulin polymerization,[6] they favor to various degrees the formation of microtubules. These results suggest a different binding site or mode of interaction for the tested compounds. Introduction of functional groups of increasing polarity at the position of the double bond of combretastatin D-2 leads to compounds of increasing activity with the more polar hydroxyl substituted derivatives being the most active. On the other hand, introduction of a bulky bromine substituent on the catecholic aromatic ring leads to diminished activity. Notwithstanding the above interesting observations, all compounds were practically inactive (IC_{50} >2500 nM) when tested against three Taxol® resistant cell lines[7] (1A9, PTX10, PTX22).

Table 1. Effect of tested compounds on tubulin polymerization.[a]

Compound	Degree of tubulin polymerization			
	0.25 mM	0.5 mM	1.0 mM	2.0 mM
Colchicine	4%	3%	4%	0%
2	16%	19%	27%	35%
3	0%	20%	27%	39%
4	5%	28%	35%	54%
5	27%	47%	62%	76%
6	3%	21%	39%	67%

[a] The assay with Epothilone B[b] (20 μM) resulted in 100% polymerization.

In conclusion, we have found that combretastatin D derivatives stabilize microtubules. This is the first indication that these compounds might exert their biological action through interaction with the tubulin polymerization machinery and, despite their inactivity against the selected cell lines, deserve further investigation.

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