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Tubulin-guided dynamic combinatorial library of thiocolchicine–podophyllotoxin conjugates

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ABSTRACT

The use of tubulin as a target to influence the composition of the mixture from a dynamic combinatorial library, based on the disulfide bond exchange reaction, is described. ESI-FT-ICR-MS was used to determine the composition of the library. The heterodimeric compound amplified by this approach was used to design the homologous derivative with a two-carbon spacer in place of the disulfide function. The ability of the compounds to inhibit tubulin polymerization is reported and compared to thiocolchicine.

1. Introduction

Tubulin and microtubules remain one of the most interesting targets in the design of new anticancer compounds.[1] In addition, increasing evidence supports a pathogenic role for tubulin in the neurodegeneration underlying several diseases, including Alzheimer's and Parkinson's disease,[2a,b] and the therapeutic potential of anti-tubulin drugs for treating neurodegeneration has been very recently proposed.[2c]

The discovery of new compounds that bind to tubulin is a challenging research aim with implications for the design and preparation of new high-quality collections of compounds useful for the study of the regulation of different ailments of modern society. The generation of divalent or multivalent compounds is a well-known strategy inspired by nature, that has produced a number of interesting results.[3] In our lab we accomplished the preparation of a number of multivalent conjugates, using as building blocks several natural products with well-documented pharmacological activity.[4]

Target-guided synthesis[5] emerged in the last decade as an important strategy that increases the versatility of combinatorial chemistry and in particular of Dynamic Combinatorial Libraries[6] (DCL), and has provided a demonstration of the evolutionary process that generates the tremendous molecular diversity of natural products.[7] In our earlier work, we have demonstrated the potential of the disulfide-exchange reaction to generate a dynamic library of thiocolchicine–podophyllotoxin adducts and the influence on the composition of this mixture induced by subtilisin or albumin.[8] Our next aim was to demonstrate the possibility of using tubulin to guide the evolutionary formation of divalent binders by favouring the structure that is thermodynamically most stabilized by non-covalent interactions with the biological target (Fig. 1).

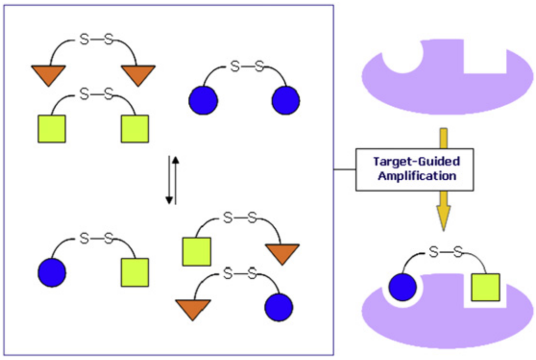

Fig. 1. General concept for the target-guided DCL based on the disulfide-exchange reaction.

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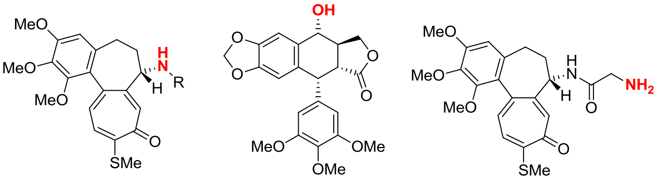
In this paper we report the use of thiocolchicine and podophyllotoxin (Fig. 2) for the generation of a dynamic combinatorial mixture of homo- and heterodimeric compounds based on the reversible formation of disulfide bonds.[9] The building blocks for the library were the homodimers obtained by condensation of thiocolchicine and podophyllotoxin with dicarboxylic acids incorporating a disulfide bond.



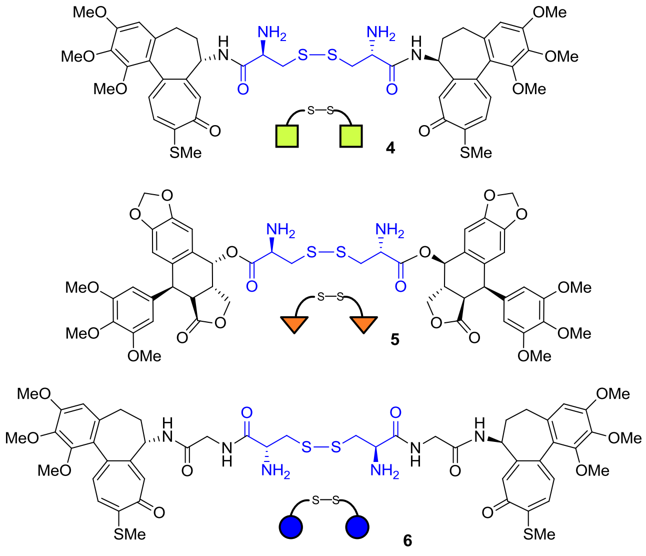
1 R = H
1a R = COCH$_3$
2
3

Fig. 2. Structure of deacetylthiocolchicine **1**, podophyllotoxin **2** and glycinoyl-deacetylthiocolchicine **3**.

Although thiocolchicine[10] and podophyllotoxin[11] recognize the same binding site, previous work in other areas has shown that homo- and heterodimers can have biological characteristics that are not merely the sum of the properties of the single entities.[3a] For this reason, we cannot predict if the interaction will be with single α- or β-tubulin, with the α-β heterodimer or with the microtubule assembly and this makes it of interest to consider conjugate compounds.

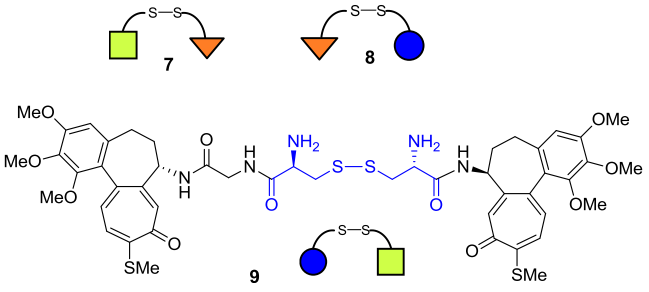
2. Results and discussion

We needed to generate a water soluble library, allowing us to work in aqueous conditions that would maintain the coherent structure of the biological target. Thus, our first task was to prepare a range of water soluble homodimeric starting compounds. We designed three different homodimers (Fig. 3) in which the spacer that connects the two entities presents primary amino and amide groups. They were obtained by condensation between podophyllotoxin **2**, deacetylthiocolchicine **1** and deacetylglycinylthiocolchicine **3** with *N*-Boc-cystine. The removal of the Boc group generated, respectively, compounds **4**,[4a] **5**[4a] and **6**, all of which had a satisfactory water solubility (1.2–1.5 mg/mL, as ditartrate salts). The three homodisulfides were mixed in a buffered solution at a concentration of 5×10^{-3} mM and maintained at room temperature for 96 h. The composition of the mixture was then evaluated by ESI-FT-ICR-MS (Supplementary data), which showed all six possible compounds due to the formation of the heterodimers **7**–**9** (Fig. 4). In parallel we prepared a second solution containing a cytoskeleton fraction to give a 2.1×10^{-6} M solution of tubulin. The analysis after 96 h revealed a marked alteration of the mixture composition (Table 1) and, in particular, we observed the amplification of the heterodimer **9**.



4
5
6

Fig. 3. Structures of compounds **4**–**6**.



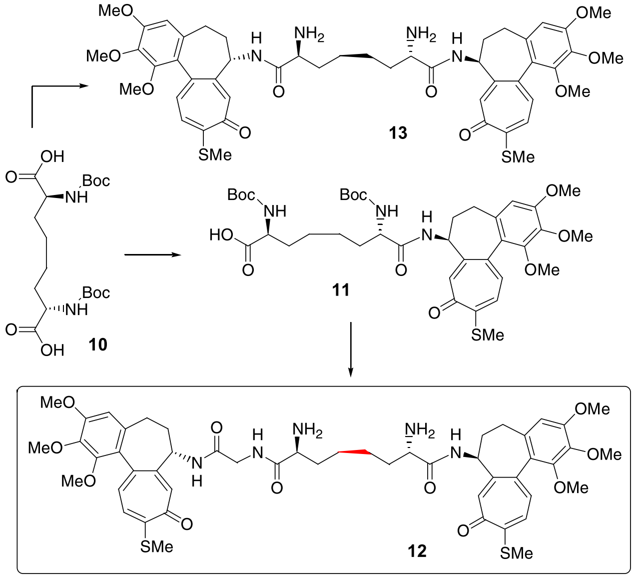
7
8
9

Fig. 4. Structures of compounds **7**–**9**.

Table 1
Comparison of the percentages of dimeric structures based on the relative intensity of the mass peaks in the presence or absence of tubulin

Compound	Mass peak	No biol. target	With biol. target
4	951.28422	58.3	37.5
5	1033.27563	3.8	—
6	1065.32572	27.6	44.6
7	992.27994	1.3	—
8	1049.29456	2.5	—
9	1008.30411	6.4	17.8

We then undertook the synthesis of compound **12** that resembles the structure of the selected compound **9** but with an ethylene group in the place of the liable disulfide bond to guarantee the binary structure in the cell medium. The condensation of the dicarboxylic acid **10**[4a] with deacetylthiocolchicine **1** gave adduct **11** that was subsequently reacted with glycinoylthiocolchicine to obtain, after Boc removal, the desired compound **12** (Scheme 1).



13
10
11
12

Scheme 1. Synthesis of compound **12**.