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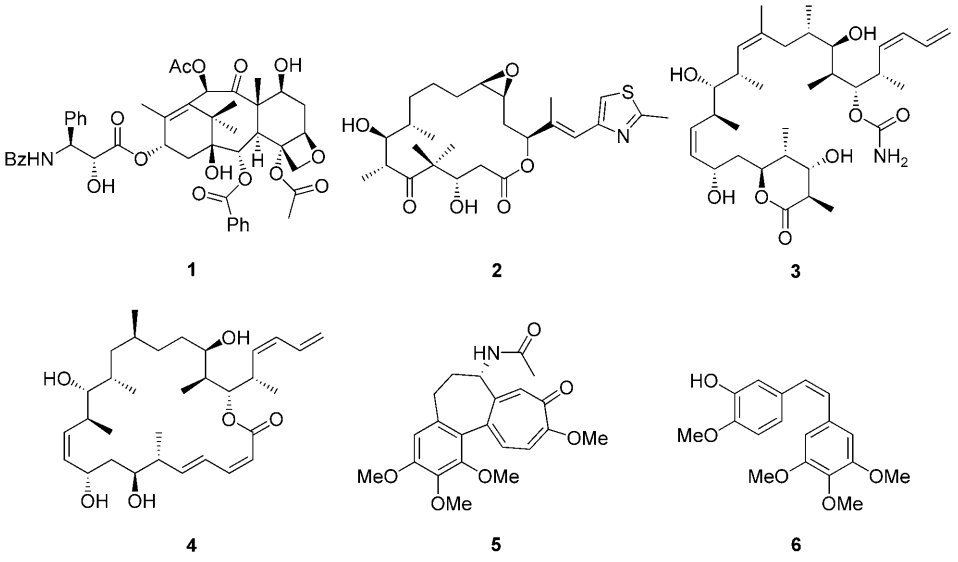
Toward Docking-Based Virtual Screening for Discovering Antitubulin Agents by Targeting Taxane and Colchicine Binding Sites

Laurent Soulère*[a, b]

Tubulin proteins play a major role in cell division, and were first identified as attractive targets for cancer therapy over 40 years ago. Recent studies aiming to gain structural insight into the binding mode of antitubulin agents to taxane or colchicine binding sites using NMR and/or molecular-mechanics-assisted conformation analysis and docking simulations have been reported. These studies, which represent the main focus of this article, create prospects for the development of docking-based virtual screening techniques to aid in the discovery of new antitubulin agents.

α,β-Tubulin heterodimers are components of both microtubules and cytoskeleton, and they are involved in crucial biological processes such as cell mobility, cell to cell interactions and mitosis.[1] Therefore, microtubules and tubulins have been recognised as attractive targets for anticancer drugs.[2] The study of paclitaxel (**1**) (taxol), epothilone A (**2**), discodermolide (**3**), dictyostatin (**4**), colchicine (**5**), combretastatin A-4 (**6**) and their derivatives, well-known inhibitors of tubulin polymerisation, has been extensively investigated.[3]



1 **2** **3** **4** **5** **6**

Competitive binding assays,[4] NMR studies,[5] and in particular docking studies have been devoted to gaining insight into the binding modes of these inhibitors, and to define the binding site of these antitubulin agents, discriminating between the taxane binding site (Figure 1 a) and the colchicine site (Figure 1 b). For this purpose, the importance of reported structures of tubulin–taxol,[6] tubulin–colchicine,[7] and tubulin–epothilone A[8] complexes is exceptional for docking studies and binding mode examination.

For example, Canales and co-workers recently reported NMR-assisted conformational analysis and docking studies of discodermolide and dictyostatin.[10] NMR spectroscopic data on these compounds in solution with or without microtubules and molecular mechanics calculations provided insight into their bioactive conformation. Molecular docking experiments were performed to corroborate the conformation search and to examine interactions between these antitubulin agents and α,β-tubulin heterodimers. All the results of this work were consistent with a binding of discodermolide and dictyostatin at the taxane site.

An interesting study of the correlation between calculated and measured free energies of binding ($\Delta G_{binding}$) of synthetic polysubstituted pyrrole compounds to α,β-tubulin has been investigated.[11] Biological evaluation of antiproliferative activity against human tumour cell lines and determination of microtubule depolymerisation activity were correlated with the calculated $\Delta G_{binding}$ values obtained from docking experiments. The relationship between calculated and measured $\Delta G_{binding}$ indicated a binding of these compounds at the colchicine site.

[a] *Dr. L. Soulère*
INSA Lyon, Laboratoire de Chimie Organique
Institut de Chimie et Biochimie Moléculaires et Supramoléculaires
Bât J. Verne, 20 av A. Einstein, 69621 Villeurbanne Cedex (France)
Fax: (+33) 472 43 88 96
E-mail: laurent.soulere@insa-lyon.fr

[b] *Dr. L. Soulère*
CNRS, UMR 5246 ICBMS, Université Lyon 1, INSA-Lyon, CPE-Lyon
Bât CPE, 43 bd du 11 Novembre 1918, 69622 Villeurbanne Cedex (France)

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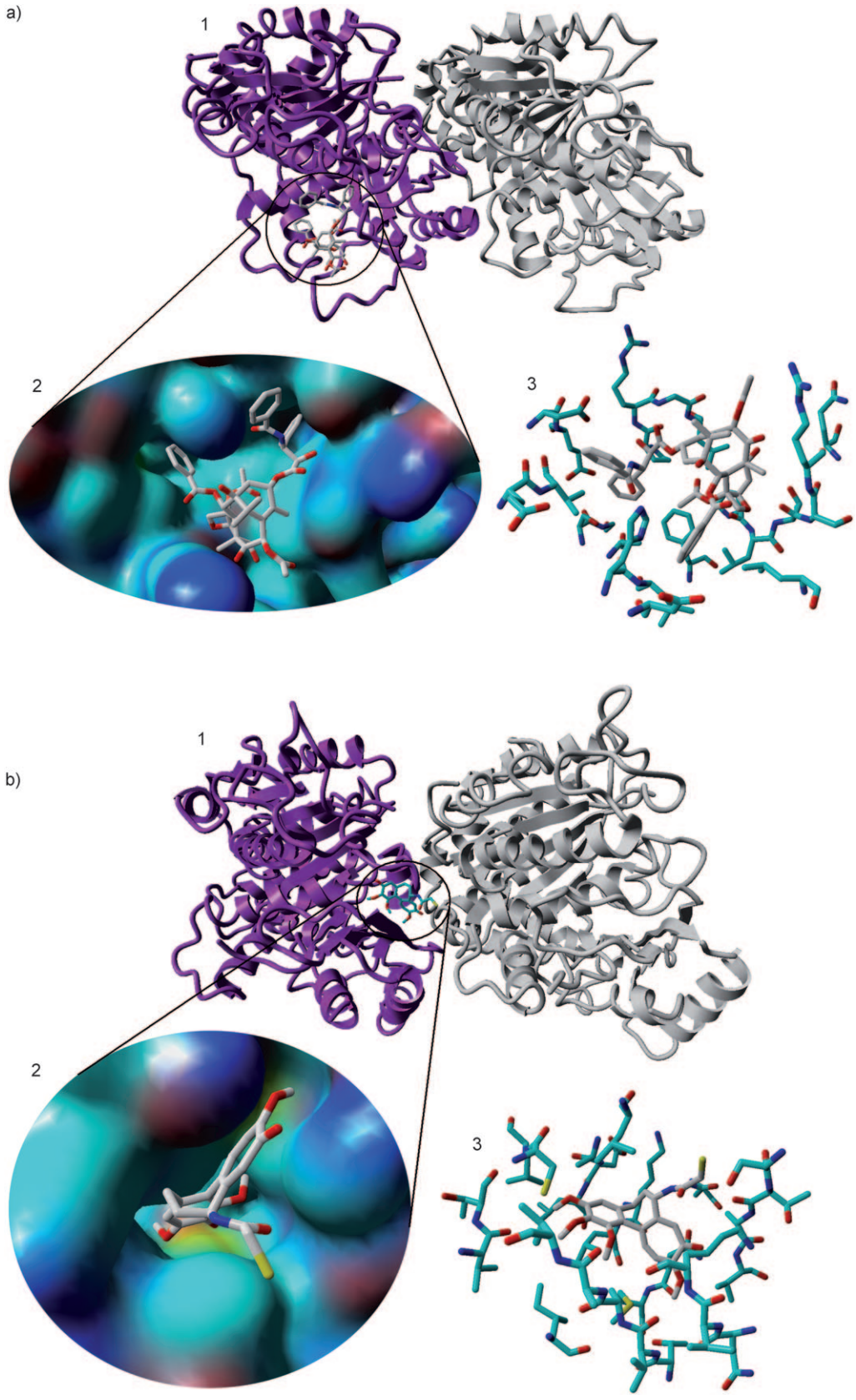

Figure 1. a) The taxane binding site: 1) α,β-tubulin heterodimer with secondary structure and with taxol bound to β-tubulin; 2) Surface representation of β-tubulin with taxol; 3) Taxane binding site overview with all residues involved in interactions with taxol. (Pictures were created using YASARA[9] from the PDB code: 1JFF[6]). b) The colchicine binding site: 1) α,β-tubulin heterodimer with secondary structure and with *N*-deacetyl-*N*-(2-mercaptoacetyl)-colchicine (DAMA-colchicine); 2) Surface representation of β-tubulin with DAMA-colchicine; 3) colchicine binding site overview with all residues involved in interactions with DAMA-colchicine. (Pictures were created using YASARA from the PDB code: 1SA0[7])

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In order to gain insight into structure–activity relationships, docking simulations have also been reported studying the binding mode of taxoid analogues.[12] Docked tubulin–ligand complexes of halogenated taxol analogues exhibited a bromine–carbonyl oxygen interaction. This observation suggested new possibilities for designing microtubule-stabilising compounds. Similar approaches have been explored in the investigation into the binding mode of triazole-, thiophene-, and imidazole-based colchine or combretastatin analogues.[13] These studies depicted structural insight into docked tubulin–inhibitors complexes obtained as results of docking experiments within the colchicine binding site.

Computer-assisted design of potential antitubulin agents using structure-based approaches involving conformation analysis and subsequent molecular docking studies have also been well described. Interestingly, these strategies provide a conformationally constrained taxol analogue, SB-T-2053,[14] and a common pharmacophore for colchicine site inhibitors useful in the design of potential antitubulin agents.[15]

Despite remaining problems,[16] docking procedures,[17] calculation of free binding energies,[18] and docking-based virtual screenings of ligand databases[19] are on the way to fulfilling their promise, and have become key methodologies in drug discovery. Recent studies using virtual screening techniques to discover antiviral drugs for avian influenza neuraminidase[20] or selective G-protein-coupled receptor antagonists[21] are absolutely fascinating examples.

As reported here, docking simulation of tubulin inhibitors is widely employed, however, to date, docking-based virtual screening that targets taxane and colchicine binding sites still remains under-explored. In the future, this approach will certainly provide new antitubulin agents for cancer therapy.

Keywords: antitubulin agents · drug discovery · inhibitors · molecular modeling · virtual screening

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