

Preface

The multifaceted conformational properties of tubulin have represented a challenge for mechanistic and structural studies of the protein for the past 20 years. The conformational equilibrium between the dimeric and polymeric form of tubulin can be controlled by different solvents (H_2O , D_2O , DMSO), cofactors (divalent ions, as for example Mg^{2+} , GTP/GDP), or small molecules. Alternatively, depending on the environment and concentration, tubulin can assemble in rings, protofilaments sheets, or even amorphous aggregates. Small organic molecules bind to tubulin at several, often flexible, sites, usually stabilizing the protein in one conformational form. All in all, the large number of structural entities that tubulin can build, together with the easy inter-conversion among them as a response to external conditions, underlies an intrinsic flexibility of the protein and an amazing capacity of rearranging the tertiary interaction network of well-defined structural domains.

In this book we review the current knowledge of the mechanistic and structural aspects of the conformation switches triggered in tubulin by small organic molecules. Despite years of research by many groups around the world, the structural basis of the mechanisms of action of many tubulin binders is still unknown. Mapping of a consistent pharmacophore at specific tubulin interaction sites is tricky, due to the fact that each binding pocket in tubulin can accommodate molecules with very diverse chemical scaffolds. The case of the microtubules stabilizing agent Epopthilone (Chapters 1, 3, 4, and 5) is exemplary: despite the presence of activity data for hundreds of derivatives, no consensus has been reached either for the bioactive conformation or for the binding mode to the protein, with two contradicting models having been proposed by Nuclear Magnetic Resonance spectroscopy (NMR, Chapter 4) and Electron Microscopy (EM, Chapter 5) studies. This contradiction might reflect the different tubulin structural forms used in the two studies or might be indicative of a constitutive problem encountered in the endeavour to describe the structural properties of a very flexible complex.

Tubulin binders are usually large and complex natural products. The difficulties and challenges encountered in the chemical synthesis of these molecules and their derivatives, with the goal of building solid structure-activity relationship (SAR) datasets, are described in the first and second chapters of the book.

The first chapter focuses on the total synthesis of macrolide-based microtubules stabilizing agents and on SAR data thereof, which have not been covered in other

reviews. The SAR data are discussed in light of the structural information available for each agent.

The second chapter focuses on the total synthesis of the marine sponge-derived polyketide discodermolide. A comprehensive survey of the synthetic chemistry efforts of several groups over a 14-year period is provided together with a comparison of the different approaches.

The third chapter describes a comprehensive study of the mechanisms of activity of microtubules stabilizing drugs. Thermodynamic, kinetic, structural and functional data on microtubules stabilizing drugs are discussed in an interdisciplinary manner to generate a “time-resolved” picture of the interaction of the drugs with different tubulin forms.

The fourth and fifth chapters review the efforts and achievements made in the characterization of the structure of the complexes of tubulin with microtubules stabilizing agents by NMR (Chapter 4) and EM (Chapter 5). Especially evident is the discrepancy of the results obtained for epothilones, where the two techniques deliver radically different structures of the bound drug. Both NMR and EM models are, however, able to explain a consistent set of SAR data. The authors of the two chapters discuss critically the advantages and limitations of each methodology.

The sixth chapter reviews the structural studies of complexes of tubulin with microtubules destabilizing agents performed by X-ray crystallography. The intelligent use of a complex of two tubulin dimers and the stathmin-like domain of the RB3 protein allows crystallization of the proteins in presence of microtubules destabilizing agents. A mechanistic model for the activity of microtubules destabilizing agents is provided on the basis of the structural results.

The last chapter is a comprehensive overview of the efforts made to understand the pharmacophore of both microtubules stabilizing and destabilizing agents by molecular modelling techniques. In the absence of converging structural information, modelling is a viable technique to study the feasibility of ligand bound conformations and pharmacophore models, bearing in mind the intrinsic problems associated with the structural flexibility of the binding site(s).

The broad range of techniques used by the authors of the book to investigate the mechanisms of the conformational control exerted on tubulin by small organic molecules is representative of the complexity of the problem. I hope that you will enjoy reading this book: those of you who are actively working in the field of tubulin-binding agents may find useful insights into the mechanisms of tubulin-binding drugs and inspiration for new experiments; those of you who are not concerned about tubulin-binding agents will appreciate this survey of the joined effort of many scientists with different expertise around the world to address the intriguing and difficult problem of conformational control in tubulin.

Tubulin-Binding Agents

Synthetic, Structural and Mechanistic Insights

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