Proteins and their Inhibitors

Inhibitors of Cyclin-dependent Kinases as Antitumor Agents

Edited by *Paul J. Smith* and *Eddy W. Yue.*

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This book reviews the discovery and development of small-molecule inhibitors of cyclin-dependent kinases (CDKs) as anticancer therapeutic agents. The CDK family of proteins has been of continuing interest as targets for the development of antiproliferative agents since their discovery in the mid-1980s. Based on their central role as "gatekeepers" of the cell cycle, and their marked dysregulation in many tumor cells, efforts to develop CDK inhibitors were initially, and have been most intensely, focused on their potential utility as anticancer therapeutics. Only recently have noncancer pathologies been active areas of research. Many notable reviews documenting progress in the oncology area of CDK research have appeared over the years. This book is distinguished from earlier reviews by its heavy weighting on medicinal chemistry aspects of the important CDK inhibitor chemotypes. The information in this book would be of high value to the medicinal chemist working in oncology, or other areas related to kinase inhibition. The chapters containing drug design rationale would also entertain the structural chemist. The book would be of lesser interest to scientists that are more centrally focused on CDK biology, clinical research, or the utility of CDK inhibitors in non-oncology areas.

The first part of this book is devoted to the background biology of CDKs. There are chapters in the early section on the biology of the cell cycle, the functional regulation of the cell cycle, and in vivo mouse models used to elucidate CDK function. There is also an anomalous chapter on CDKs and vascular disease; although interesting, the chapter seems quite misplaced in a book centered on antitumor agents. The second section includes chapters on evaluating CDK inhibitor selectivity and the use of structural chemistry for inhibitor development. Despite high-throughput screening playing a key role in the initial identification of inhibitor chemotypes, the early solution of inhibitor-bound protein structures contributed significantly to the understanding and refinement of protein binding and selectivity. It has provided a fertile and entertaining testing ground for rational drug design, as documented in this section and included in other chapters. The bulk of the book, however, is devoted to the discovery and development of ten CDK inhibitor chemotypes, including the clinically studied agents: flavopiridol, R-roscovitine and BMS-387032 (recently licensed by Bristol-Myers to Sunesis, and subsequently designated as SNS-032). A detailed chapter, oriented towards medicinal chemistry, is devoted to each chemotype with the exception of flavopiridol and UCN-01. These agents are presented from a more clinical perspective. Flavopiridol is the clinically most studied of these agents, and continues to provide some of the most interesting clinical results. Although it can be found elsewhere, it would have reinforced the focus of the book if there was additional medicinal chemistry detail devoted to these pioneering agents. It would have also enhanced the reader's experience if there was a short introductory chapter at the beginning of this section devoted to an overview of the different agents, which provided a perspective of their relative historical importance. This would have been an aid prior to reading the more detailed accounts, especially if written objectively by the editors. Finally, the last portion of the book is concerned with the clinical status and yet to be fully uncovered clinical utility of these agents. This section includes combination studies (preclinical and clinical) and a perspectives chapter for future directions for the development of CDK inhibitors. As a minor point, CGP60474 is incorrectly depicted with the Bristol–Myers pyazolopyridine ring system in this final chapter.

The overall organization of the book is readily discernible through the table of contents. A preface is provided that relates the thoughts of the editors on the book's organization. The preface is informative reading prior to delving into the individual chapters. The index is extensive, although it does not include company or author names. Finally, the book would have been more timely if there was an updated section, or editor's note, written closer to press time, which included some the latest preclinical and clinical studies with CDK inhibitors. The encouraging, emerging results in B-cell lymphomas, published recently, could have been included in this section. For these the reader will have to refer to the current literature.

Disclaimer: The views expressed in this book review are those of the author, and do not represent the views of, nor are they an endorsement by, the US Government or the National Institutes of Health.

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Telomerase Inhibition: Strategies and Protocols

Edited by *Lucy G. Andrews* and *Trygve O. Tollefsbol.*

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The telomeres of human cells protect chromosomal ends from fusion events.

In human somatic cells, telomere length decreases with each cell division event; if active, a telomere maintenance mechanism increases cellular replicative capacity. Telomere length is maintained by a specialized enzyme called telomerase. The catalytic subunit of the human telomerase enzyme, a protein called hTERT, uses the RNA subunit (called hTR or hTERC) as a template and adds 5′-GGTTAG-3′ repeats to the ends of chromosomes. As demonstrated more than a decade ago (Kim et al., *Science* **1994**, *266*, 2011–2015), and as mentioned in this book's preface, telomerase activity is robust in about 90% of cancer cells, whereas this enzyme is expressed at very low levels in normal cells. Thus, the inhibition of telomerase, or its associated proteins, should result in cancer cell death with little or no effect on normal cells. This has stimulated a new area of research, and various strategies have been envisioned to inhibit telomerase. This book will provide researchers with a variety of tools to achieve that goal. Given the thousands of articles published each year with the keyword "telomerase", this summary of the methods related to the analysis of telomerase is welcome. This book contains 14 chapters by active players in the telomerase field. It starts with an introduction that summarizes the various strategies presented in subsequent chapters.

Chapters 2–8: the catalytic subunit hTERT. Chapter 2 describes the use of antisense oligonucleotides to inhibit the activity of hTERT, and Chapters 3–5 discuss hTERT inhibition using RNA-interference-related strategies. RNAi effects are transient when double-stranded RNA is introduced into the cells because the inhibitory siRNA is eventually degraded (Chapter 3). The use of plasmid vectors (Chapter 4) or retroviruses (Chapter 5) that express shRNA allows long-term gene knock-down. Depending on the type of response sought (transient or long-lasting), one may choose either protocol for hTERT inhibition.

The main focus of Chapter 6 is a detailed description of experimental protocols for flow-FISH, Q-FISH, Western blot, and cytogenetic experiments. One of the very few catalytic inhibitors found so far, BIBR 1532 (a mixed-type noncompetitive inhibitor), is described in Chapter 6. It is disappointing that this compound, like many other effective small-molecule telomerase inhibitors, has not been made commercially available. Rather unfortunately, no specific chapter on nucleoside analogues was necessary. Nucleoside analogues such as azidothymidine (AZT) act as chain-terminating inhibitors of reverse transcriptases and were among the first drugs to be tested for their ability to inhibit telomerase. Despite initial hopes, there have been no recent advances in the use of nucleoside analogues, which explains why this approach is not addressed here.

Chapter 7 is dedicated to a description of the telomerase immunization strategy, by far the most advanced clinically. In this strategy, patients are immunized with peptides found in the telomerase protein subunit; the telomerase-specific cytotoxic T-lymphocytes generated should specifically destroy cancerous cells that express telomerase. Phase III trials started in 2006 with one of these peptides (reviewed in Vonderheide, *Biochimie* **2008**, *90*, 173–180). This is a very useful chapter for those interested in hTERT peptides as tumor antigens.

Chapter 8 describes screening methods for telomerase activity modulators using an hTERT promoter fused to reporter genes (such as GFP and SEAP). This assay allows identification of compounds that interfere with hTERT expression.

Chapters 9 and 10: the RNA component (hTR or hTERC). The RNA component of telomerase, called hTR or hTERC, is necessary for telomerase reverse transcription. As hTR is a nucleic acid, it is a good target for oligonucleotide inhibitors. These oligonucleotides should be considered as "template agonists" rather than true antisense agents, as their targets are reverse transcribed rather than translated into peptides. Antisense and ribozyme strategies are treated in Chapters 9 and 10, respectively. Of particular interest are 2′,5′ oligoadenylate antisense oligomers that activate Rnase L. Geron's anti-hTR thiophosphoramidate oligonucleotides GRN163 and GRN163L are not discussed in detail, even though GRN163L is currently in clinical trials. These thiophosphoramidate oligonucleotides are not commercially available to the research community.

Chapters 11 and 12: other proteins and signaling pathways. Tankyrase 1, the telomeric poly(ADP-ribose) polymerase is overexpressed in cancer cells and confers resistance to telomerase inhibitors. This enzyme could be the target of an anticancer agent, and Chapter 11 describes a clever cell-based assay that may lead to the identification of specific inhibitors. Chapter 12 illustrates a strategy aimed at targeting the MAP kinase pathway, one of the major activators of hTERT transcription. This strategy is indirect, and the use of MAP kinase inhibitors might elicit effects in addition to telomerase inhibition. Despite this caveat, targeting of signaling pathways as a route to telomerase inhibition should be explored.

Chapter 13: activity assays. This chapter describes methods for the analysis of telomerase activity and telomere length (TRAP assay, TRF). These methods can be used for discovery and to evaluate mechanisms of telomerase inhibitors. Recently, a very elegant method termed *Telospot* was proposed for the discovery and characterization of telomerase modulators in vitro (Cristofari et al., *Nature Biotechnology* **2007**, *4*, 851–853); unfortunately, due to the publication date, this methodology was not included in this book.

Combination chemotherapy. Finally, Chapter 14 deals with telomerase inhibition in combination with other chemotherapeutic reagents. For in vivo assays and clinical trials, some authors have proposed that telomerase inhibitors should only be used to complement (or in combination with) a direct cytotoxic agent. This paradigm is suggested based on the long delay expected between the start of the treatment with telomerase inhibitors and proliferation arrest; thus these agents alone are expected to be inefficient. This drawback has been verified for some inhibitors such as BIBR 1532. Some studies have demonstrated that the inhibition or alteration of telomerase is effective against cancer only as a polytherapy in the adjuvant setting.

Clearly, it would have been a difficult challenge to cover all aspects of this in-

teresting research area, and some fields related to telomerase inhibition are not covered in this book. There is a certain imbalance in the dedication of seven chapters to hTERT and only two each to hTR and other telomeric components. Another shortcoming is the near total neglect of quadruplex ligands, which were initially designed as indirect telomerase inhibitors. However, recent studies have demonstrated that these compounds are more likely to interfere with telomeric functions and induce uncapping than act as true telomerase inhibitors. Therefore, such an absence is not detrimental. Finally, the index, despite being only three pages long, is sufficiently inclusive to be helpful.

Overall this book will be a useful resource for researchers performing telomerase-related experiments. Anticancer approaches directed at telomerase inhibition are varied, and the choice of a strategy depends on the goal. This book features a compendium of methods and provides the researcher with a set of practical tools, as all protocols are described in a very clear and accurate fashion. We would have appreciated additional figures to outline complex experimental procedures. As a final warning, one should keep in mind that this book is part of a "Methods" series and does not claim to be an introduction to the field for those having an academic interest in telomerase. Only two pages—in the first chapter—are actually dedicated to a description of the enzyme and its function. Excellent reviews or special issues can be found in the literature that do serve this purpose. This book constitutes an excellent choice *only* if the reader plans to perform telomerase inhibition-related experiments.

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Computational and Structural Approaches to Drug Discovery: Ligand–Protein Interactions

Edited by *Robert M. Stroud* and *Janet Finer-Moore*.

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Based on the substantial evolution of computational methods (bioinformatics and cheminformatics) as well as methods of structure determination (crystallography and NMR spectroscopy) during the last few decades, there has been a massive increase in the number of publications applying such methods to drug discovery. Moreover, various combinations of these methods have quickly gone beyond the level of academic exercise and have been transformed into innovative drug-producing techniques with great relevance and industrial application. The topics covered in this book comprise a historical perspective of how these methods have been developed, an assessment of their successes and failures, and prospects as to how these methods can be improved.

The editors have assembled a highly interesting team of experts from both academia and industry including many scientists who have significantly influenced and shaped the fields of research on which they report and comment in this book. In Section One, the editors themselves, together with J. Blaney, give an overview of the topic, describing "the promise and the problem". Instead of indulging themselves in praising the methods described, they assess both success and failures, and thus establish a level of open and impartial discussion that reflects the high quality and fundamental credibility of the book. Consequently, the focus on the current limitations of a given method and how they can be overcome is a strong aspect of this book. This initial overview in the first section is complemented by a retrospective given by H. Kubinyi, a medicinal chemist with decades of experience in industrial drug discovery. "The changing landscape in drug discovery" describes how new strategies and techniques have evolved and how the bottlenecks and problems have shifted accordingly. Although high initial enthusiasm for the potential of novel methods has quite often been tempered, their practical value to facilitate drug discovery becomes clear upon posing the question: "Where would we stand without them?"

Structure-based design methods are described and illustrated, as well as critically evaluated in Section Two. The successful design of purine nucleoside phosphorylase (PNP) inhibitors is presented by Y. Zhang and S. E. Ealick to exemplify the usefulness of high-resolution crystal structures in drug design. In the following chapter, A. M. Davis, S. J. Teague, and G. J. Kleywegt describe applications and limitations of X-ray crystallographic data, such as hidden uncertainties when deriving and depositing an atomic model from the electron density. This chapter is especially valuable for modelers without personal experience in crystallography and a warning for all who use crystal structure coordinates uncritically as the "ultimate truth". A fundamental problem, mentioned above, is presented in detail (Chapter 5) by D. Hamelberg and J. A. McCammon, in their discussion about bound waters: "Do they leave or stay?" They report on the usefulness of computational methods for predicting the positions of bound waters in protein cavities and discuss the impact of such bound waters on the free energy of ligand binding. More importantly, they relate this insight to strategies that try to harvest these effects in drug discovery. In Chapter 6, M. L. Verdonk and W. T. M. Mooij explicate how the steadily increasing amount of crystallographic data on protein–ligand complexes has lead to the development of knowledge-based techniques by deriving statistical preferences for the interaction between atoms or functional groups. These methods have gained importance in structure-based design by predicting "binding hotspots" or as scoring functions such as DrugScore, PMF, and ASP. The authors critically assess that these may not have been proven superior to empirical or force-field-based scoring functions in general, but they provide great potential for the implementation of tailor-made scoring functions for particular targets or target families. Con-

