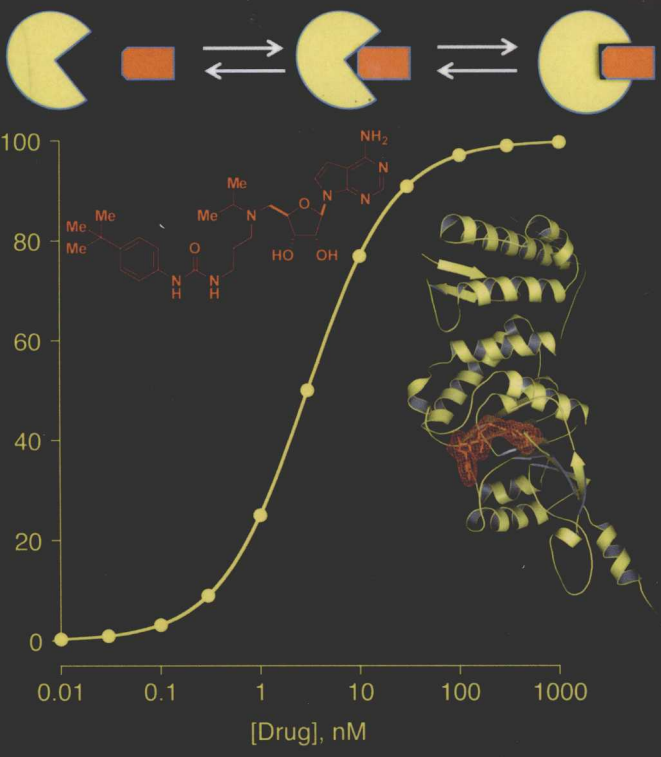
Evaluation of Enzyme Inhibitors in Drug Discovery

A Guide for Medicinal Chemists and Pharmacologists

With a Foreword by
Christopher T. Walsh, Harvard Medical School

SECOND EDITION



Robert A. Copeland

WILEY

EVALUATION OF ENZYME INHIBITORS IN DRUG DISCOVERY

A Guide for Medicinal Chemists and Pharmacologists

Second Edition

ROBERT A. COPELAND

WILEY

A JOHN WILEY & SONS, INC., PUBLICATION

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data:

Copeland, Robert Allen.
Evaluation of enzyme inhibitors in drug discovery: a guide for medicinal chemists and pharmacologists / by Robert A. Copeland. – 2nd ed.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-1-118-48813-3 (hardback)
I. Title.
[DNLM: 1. Enzyme Inhibitors–therapeutic use. 2. Drug Design. 3. Enzyme Inhibitors–chemistry. QU 143]
615'.19–dc23

2012045179

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

EVALUATION OF ENZYME INHIBITORS IN DRUG DISCOVERY

To the three bright stars of my universe: Nancy, Lindsey, and Amanda.

FOREWORD TO SECOND EDITION

Evalaution of Enzyme Inhibitors in Drug Discovery by Robert A. Copeland has been an invaluable guide to the community of scientists devoted to finding new drugs in all therapeutic areas of human disease since the publication of the first edition in 2005. The text has been a key guide both for academic groups interested in identification of new druggable targets and for medicinal chemistry and pharmacology teams in biotechnology and pharmaceutical laboratories. Its centrality is due to the clarity of presentation on how enzymes work, how substrates and inhibitors are bound, and what forces contribute to selectivity and strength of binding of both types of ligands. Because enzymes are both intracellular and extracellular targets, including knases, phosphatases and proteases, as well as the catalysts for group transfers of methyl, acetyl, glycosyl, and ubiquitinyl groups to and from protein substrates, the principles and applications in this book are relevant in all major therapeutic drug classes.

Dr. Copeland has a deep scholarly grasp of the kinetic and thermodnamic aspects of ligand-protein interactions and the catalytic steps that can ensue, and he repeatedly frames them with current examples of practical utility. More than in any other treatment of enzyme kinetics and catalysis, these subjects are accessible to drug hunters. Both the hows and whys of assay design, implementation, and analyses are set forth, from configuration of initial screens to lead optimization efforts in vitro and selection of lead structures from in vivo pharmacology.

Discovery and optimization of enzyme inhibitors that can get to and through clinical trials requires understanding of classical and nonclassical modes of inhibitor binding, how to measure them, and why one class may be preferred for a specific target group. Particularly interesting for drug discovery efforts

are molecules that, in their interactiion with target proteins, show affinities that fall in the regime of slow binding, tight binding, and slow, tight binding categories, arriving at subnanomolar levels of affinity. In the limit of tight binding inhibitors are those molecules that act irreversibly through covalent bond formation; as part of this treatment, Copeland notes some "quiescent affinity labels" that appear to be moderately electrophilic and so offer the promise of target protein selectivity.

The second edition updates the treatment of the relevant modes of inhibition with contemporary literature examples that illustrate pitfalls to be avoided and kinetic analyses that allow lead optimizations to be more efficient. Chapters 8, "Drug-Target Residence Time," and 10, "Quantitative Biochemistry in the Pharmacological Evaluation of Drugs," in this edition are totally new and are substantive advancements. Copeland has been a leader in demonstrating the theoretical and practical utility of using the drug residence time ($\tau = 1/k_{off}$) rather than the dissociation constant K_D to explain why drug–target protein lifetime and not just measurement of inhibitor affinity is a valuable, predictive correlate of "durable pharmacological effects." In whole animals, drugs that have long τ values can have desirable pharmacodynamics not otherwise predicted by systemic pharmacokinetics. The clear value of these concepts in lead optimization for drugs and clinical candidates is illustrated with several recent examples for small molecules, peptides, and antibodies as ligands.

The second edition concludes with the new chapter on how absorption, distribution, metabolism, and excretion, the ADME core of in vivo pharmacology, can be factored into quantitative biochemical parameters that are equivalent to the tools and concepts developed throughout the text. While this chapter is not meant to be a substitute for a full pharmacology text, it demystifies many of the terms, assays, measurements and analyses of classical pharmacology, setting them into the same framework of kinetic and thermodynamic measurements familiar to investigators who conduct biochemical assays. These include classical pharmacologic measurements of pharmacokinetic half-lives, stability of drugs in plasma, plasma protein binding, hepatic metabolism with attention to the plethora of cytochromes P450, clearance rates, AUC measurements, allometric scaling, target occupancy calculations, and hERG channel monitoring.

The second edition of *Evaluation of Enzyme Inhibitors in Drug Discovery* should increase the probability of success for any of its serious readers.

CHRISTOPHER T. WALSH

Department of Biological Chemistry and Molecular Pharmacology
Harvard Medical School

PREFACE TO SECOND EDITION

In the seven years since the publication of the first edition of this text, there has been a continued expansion of interest in the quantitative evaluation of enzyme inhibitors for the applied sciences of drug discovery and drug development. While this time period has seen some contraction of R&D efforts in large pharmaceutical companies, there has also been a compensatory expansion of efforts in academic, government, and smaller biopharmaceutical facilities across the globe. The need for rigorous, quantitative evaluation of drug–target interactions remains paramount to these endeavors, and a broader appreciation for the power that quantitative biochemistry brings to drug discovery applications has emerged.

Thus, it seems timely to update and expand the coverage of these important topics in a second edition of this book. In the pages to follow, I have attempted to improve upon the first edition by substantially expanding most of the chapters with two overarching aims: to cover more completely the experimental aspects of the evaluation methods contained in each chapter and to enhance the clarity of the presentation, especially for the newcomer to applied enzymology. Toward these ends, a number of additional appendices have been added to the text, providing ready sources of useful information as they apply to quantitiative biochemistry in drug discovery.

I have also added two new chapters to this second edition. The first of these presents in detail the concept of drug–target residence time. This concept posits that the key driver of durable, in vivo pharmacology is not the affinity of a drug for its intended target per se, but rather the lifetime of the binary drug–target complex. I present arguments to suggest that the in vivo lifetime of the drug–target complex can be directly correlated with the residence time

of the complex, which is defined as the reciprocal of the dissociation rate constant (k_{off}). Details of how k_{off} and residence time can be quantified through in vitro measurements during lead optimization are presented.

The development of enzyme inhibitors as therapeutic agents involves optimization of multiple pharmacologic properties beyond the affinity and selectivity of the molecule for its target enzyme. Many of these pharmacologic properties have their molecular underpinning in biochemical reactions within the human body. Examples of this include drug absorption from the gastrointestinal tract via active and passive transport mechanisms, metabolic clearance of drugs from systemic circulation, hepatic and renal drug metabolism, and adverse effects mediated by drug interactions with off-target enzymes, ion channels, and receptors. Thus, the second new chapter of this text presents an overview of the role of quantitative biochemistry in the pharmacological evaluation of drug molecules during preclinical development. The reader will see that much of drug discovery and development can be understood in terms of the same quantitative biochemical principles that guide the in vitro evaluation of enzyme inhibitor affinity, binding kinetics and selectivity as presented in the earlier chapters of this book. Hence, as with the first edition of this book, my aim here is to provide a readable introduction to the guiding principles and experimental methods for evaluation of enzyme inhibitors throughout the drug discovery and development process, for readers of diverse scientific backgrounds, including medicinal chemists, biochemists, biologists, pharmacologists, and physicians. Thus, the intention here is not to present an advanced text on enzymology for the seasoned practitioner, but rather to put the science of enzymology into the broader context of drug discovery and development and to develop the underlying concepts in such a way as to be understood and appreciated by students and professionals across the broad expanse of scientific skill bases required for successful drug discovery.

As with the first edition, I have been greatly aided in the development of the current text by numerous interactions with colleagues, students, and friends. Beyond those acknowledged in the first edition, I would like to thank the employees of Epizyme, Inc., the Novartis Institute for Biomedical Research (Basel, Switzerland), Agios Pharmaceuticals, and Infinity Pharmaceuticals; and students at the Broad Institute of Harvard University and M.I.T. for their contributions to courses in applied enzymology that I have taught and that have helped to refine the presentations within this new edition. I would also like to thank Robert Gould, Jason Rhodes, Mikel Moyer, Victoria Richon, Margaret Porter Scott, Aravind Basavapathruni, Kevin Kuntz, David Swinney, and Paul Pearson for helpful comments and stimulating conversations that added to the text in many ways. I am also grateful to Ms. Caroline Hill and Ms. Kristy Maniatis, who generously aided me in gathering and collating some reference materials. Professor Christopher T. Walsh of Harvard Medical School has long been a source of great inspiration and mentoring, as well as a cherished friend. I thank him for all of his help and advice through the years and

for agreeing to write the foreword for this second edition. Finally, as always, my greatest source of inspiration, affirmation, pride, love, laughter, and great fun is my family. I thank my incredible wife Nancy and our amazing daughters Lindsey and Amanda for their constant support, patience, and love.

ROBERT A. COPELAND

FOREWORD TO FIRST EDITION

Evaluation of Enzyme Inhibitors in Drug Discovery: A Guide for Medicinal Chemists and Pharmacologists is a valuable reference work that clearly addresses the need for medicinal chemists and pharmacologists to communicate effectively in the difficult and demanding world of drug discovery. During the 20th century, the pharmaceutical industry evolved into a large, complex, international endeavor focused on improving human health largely through drug discovery. Success in this endeavor has been driven by innovative science that has enabled discovery of new therapeutic targets, biological mechanisms of drug action for approaching these targets, and chemical entities that operate by these mechanisms and are suitable for clinical use. Modulators of receptor function and enzyme inhibitors have been central to this discovery process. As the industry evolved, so did the relative importance of enzyme inhibitors. For many years, treatment of hypertension was dominated by modulators of receptor function such as beta blockers and calcium antagonists. The discovery of orally active angiotensin converting enzyme inhibitors shifted the balance of treatment modalities towards enzyme inhibitors for this common disease in the late 1970s and early 1980s. Similarly, the dominant treatment for high cholesterol level now is an HMG-CoA reductase inhibitor popularly referred to as a "statin." Thus, it is clear that a thorough understanding of enzymology is a necessary tool for medicinal chemists and pharmacologists to share as they pursue the complex goals of modern drug discovery. The large number of kinases, phosphatases, and protein processing enzymes that can currently be found on many drug discovery agendas emphasizes this point.

In *Evaluation of Enzyme Inhibitors in Drug Discovery*, Robert A. Copeland brings clarity to the complex issues that surround understanding and

interpretation of enzyme inhibition. Key topics such as competitive, noncompetitive, and uncompetitive inhibition; slow binding and tight binding; and the use of Hill coefficients to study reaction stoichiometry are discussed in language that removes the mystery from these important concepts. Many examples of each concept can be found in the discussions, with emphasis on the clinical relevance of the concept and on practical application that does not short-change an understanding of underlying theory. The necessary mathematical treatments of each concept are concisely presented with appropriate references to more detailed sources of information. Understanding the data and the experimental details that support it has always been at the heart of good science and the assumption-challenging process that leads from good science to drug discovery. This book helps medicinal chemists and pharmacologists to do exactly that in the realm of enzyme inhibitors. In short, this is a very readable book that admirably addresses the purpose set forth in the title.

PAUL S. ANDERSON, Ph.D.

Vice President of Chemistry, Merck Research Laboratories (retired)

PREFACE TO FIRST EDITION

Enzymes are considered by many in the pharmaceutical community to be the most attractive targets for small molecule drug intervention in human diseases. The attractiveness of enzymes as targets stems from their essential catalytic roles in many physiological processes that may be altered in disease states. The structural determinants of enzyme catalysis lend themselves well to inhibition by small molecular weight, drug-like molecules. As a result, there is a large and growing interest in the study of enzymes with the aim of identifying inhibitory molecules that may serve as the starting points for drug discovery and development efforts.

In many pharmaceutical companies, and increasingly now in academic laboratories as well, the search for new drugs often starts with high throughput screening of large compound libraries. The leads obtained from such screening exercises then represent the starting points for medicinal chemistry efforts aimed at optimization of target affinity, target selectivity, biological effect, and pharmacological properties.

Much of the information that drives these medicinal chemistry efforts comes from the in vitro evaluation of enzyme-inhibitor interactions. Enzymes are very often the primary molecular targets of drug-seeking efforts; hence, target affinity is commonly quantified using in vitro assays of enzyme activity. Likewise, the most obvious counterscreens for avoidance of untoward side effects are often enzyme activity assays. Metabolic transformations of xenobiotics, including most drug molecules, are all catalyzed by enzymes. Therefore, careful, quantitative assessment of compound interactions with metabolic enzymes (e.g., the cytochrome P450 family) is an important component to compound optimization of pharmacokinetic properties.

Thus, while screening scientists and enzymologists are typically charged with generating quantitative data on enzyme-inhibitor interactions, it is the medicinal chemists and biological pharmacologists who are the ultimate "customers" for these data. It is therefore imperative that medicinal chemists and pharmacologists have a reasonable understanding of enzyme activity and the proper, quantitative evaluation of the interactions of enzymes with inhibitory molecules, so that they may use this information to greatest effect in drug discovery and optimization. Over the past several years, I have been invited to present courses on these topics to medicinal chemistry groups and others at several major pharmaceutical companies. It is apparent that this community recognizes the importance of developing a working knowledge of enzyme-inhibitor interactions and of quantitative, experimental evaluation of these interactions. The community likewise has expressed to me a need for a textbook that would provide the colleagues of biochemists and screening scientists—the medicinal chemists and pharmacologists—with a working knowledge of these topics. This is the aim of the present text.

There are many enzymology texts available (my own previous text included) that provide detailed information on enzymology theory and practice, and are primarily aimed at biochemists and others who are directly involved in experimental studies of enzymes. In contrast, the aim of the present text is to provide chemists and pharmacologists with the key information they need to answer questions such as: What opportunities for inhibitor interactions with enzyme targets arise from consideration of the catalytic reaction mechanism? How are inhibitors properly evaluated for potency, selectivity, and mode of action? What are the potential advantages and liabilities of specific inhibition modalities with respect to efficacy in vivo? And finally, what information should medicinal chemists and pharmacologist expect from their biochemistry/enzymology colleagues in order to most effectively pursue lead optimization? In the text that follows I attempt to address these issues.

The text begins with a chapter that describes the advantages of enzymes as targets for drug discovery and some of the unique opportunities for drug interactions that arise from the catalytic mechanisms of enzymes. We next explore the reaction mechanisms of enzyme catalysis (Chapter 2) and the types of interactions that can occur between enzymes and inhibitory molecules that lend themselves well to therapeutic use (Chapter 3). Two chapters then describe mechanistic issues that must be considered when designing enzyme assays for compound library screening (Chapter 4) and for lead optimization efforts (Chapter 5), respectively. The remainder of the book describes proper analysis of special forms of inhibition that are commonly encountered in drug-seeking efforts, but that can be easily overlooked or misinterpreted. Hence, the book can be effectively utilized in two ways. Students, graduate-school course directors, and newcomers to drug discovery research may find it most useful to read the book in its entirety, relying on the first three chapters to provide a solid foundation in basic enzymology and its role in drug discovery. Alternatively, more experienced drug discovery researchers may chose to use

the text as a reference source, reading individual chapters in isolation, as their contents relate to specific issues that arise in the course of ongoing research efforts.

The great power of mechanistic enzymology in drug discovery is the quantitative nature of the information gleaned from these studies, and the direct utility of these quantitative data in driving compound optimization. For this reason, any meaningful description of enzyme-inhibitor interactions must rest on a solid mathematical foundation. Thus, where appropriate, mathematical formulas are presented in each chapter to help the reader understand the concepts and the correct evaluation of the experimental data. To the extent possible, however, I have tried to keep the mathematics to a minimum, and instead have attempted to provide more descriptive accounts of the molecular interactions that drive enzyme-inhibitor interactions.

Thus, the aim of this text is to provide medicinal chemists and pharmacologists with a detailed description of enzyme-inhibitor evaluation as it relates directly to drug discovery efforts. These activities are largely the purview of industrial pharmaceutical laboratories, and I expect that the majority of readers will come from this sector. However, there is an ever-increasing focus on inhibitor discovery in academic and government laboratories today, not only for the goal of identifying starting points for drug development, but also to identify enzyme inhibitors that may serve as useful tools with which to understand better some fundamental processes of biological systems. Hence, graduate and post-graduate students and researchers in these sectors may find value in the current text as well.

ROBERT A. COPELAND

ACKNOWLEDGMENTS (From First Edition)

There are many friends and colleagues who have contributed in different ways to the development of this text. The need for a book on evaluation of enzyme inhibition in drug discovery was made clear to me by two individuals: David L. Pompliano and Robert A. Mook, Jr. I am grateful to both of them for inspiring me to write this book. I also benefited from the continuous encouragement of John D. Elliott, William Huffman, Allen Oliff, Ross Stein, Thomas Meek, and many others. Stimulating conversations with Trevor Penning, Dewey McCafferty, David Rominger, Sean Sullivan, Edgar Wood, Gary Smith, Kurt Auger, Lusong Luo, Zhihong Lai, John Blanchard, and Benjamin Schwartz also helped to refine my thoughts on some of the concepts described in this book. I have imposed on a number of colleagues and friends to read individual chapters of the text, and they have graciously accommodated these requests and provided thoughtful comments and suggestions that have significantly improved the content of the book. I am grateful to Zhihong Lai, Lusong Luo, Dash Dhanak, Siegfried Christensen, Ross Stein, Vern Schramm, Richard Gontarek, Peter Tummino, Earl May, Gary Smith, Robert Mook, Jr., and especially to William J. Pitts who read the entire manuscript and offered many valuable suggestions. I am also indebted to Paul S. Anderson for reading the manuscript and graciously agreeing to write the foreword for this book, and for the guidance and advice he has given me over the years that we have worked together. Neysa Nevins was kind enough to provide several illustrations of enzyme crystal structures that appear in the text. I thank her for helping me with production of these figures. I would also like extend my thanks to the many students at the University of Pennsylvania School of Medicine, and also at the Bristol Myers Squibb and GlaxoSmithKline Pharmaceutical

Companies, who have provided thoughtful feedback to me on lectures that I have given on some of the topics presented in this book. These comments and suggestions have been very helpful to me in formulating clear presentations of the sometimes complex topics that needed to be covered. I also thank the editorial staff of John Wiley & Sons, with whom I have worked on this and earlier projects. In particular, I wish to acknowledge Darla Henderson, Amy Romano, and Camille Carter for all their efforts. Finally, and most importantly, I wish to thank my family, to whom this book is dedicated: my wife, Nancy, and our two daughters Lindsey and Amanda. They are my constant sources of love, inspiration, energy, encouragement, insight, pride, and fun.

R. A. C.