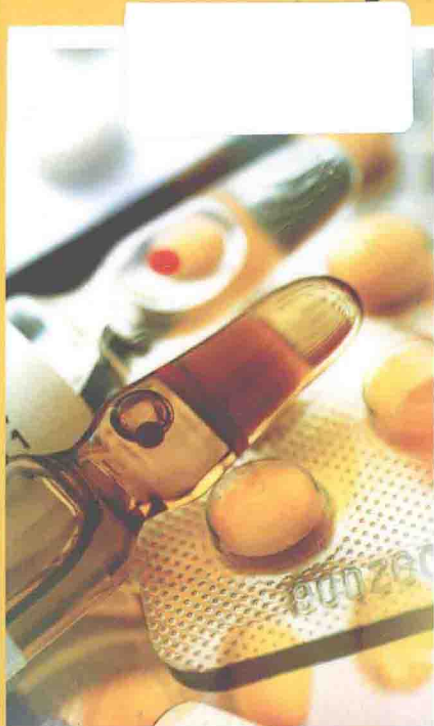


Basic Principles of Drug Discovery and Development



Benjamin E. Blass



BASIC PRINCIPLES OF DRUG DISCOVERY AND DEVELOPMENT

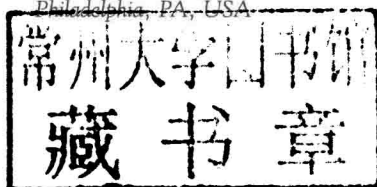
BENJAMIN E. BLASS

Temple University School of Pharmacy

Moulder Center for Drug Discovery Research

North Broad Street

Philadelphia, PA, USA



AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEWYORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier



Academic Press is an imprint of Elsevier
32 Jamestown Road, London NW1 7BY, UK
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA
225 Wyman Street, Waltham, MA 02451, USA
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

Copyright © 2015 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

ISBN: 978-0-12-411508-8

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

For information on all Academic Press publications
visit our website at <http://store.elsevier.com/>

Printed and bound in the USA



**Working together
to grow libraries in
developing countries**

www.elsevier.com • www.bookaid.org

BASIC PRINCIPLES OF DRUG DISCOVERY AND DEVELOPMENT

Dedication

Sir Isaac Newton, one of the greatest scientists of his time, wrote “If I have seen further it is by standing on ye shoulders of Giants.” Although he was almost certainly referring to his scientific achievements, the underling concept of learning from our forbearer is true in any endeavor. Indeed, this concept can be further extended to include those who are there in the present day, supporting the activities of an individual as he or she attempts to accomplish that which they view as important. With this thought in mind, I have dedicated this book to the scientists who came before me, those who mentored me, and those who work with me on a daily basis. In addition, and perhaps more importantly, this text is dedicated to the loving and supportive family that has helped me become the person that I am today. Special thanks are offered to my mother, father, sister, brother, my three children, and of course, my wife Kathleen. These are the giants on whose shoulders I have stood upon.

Foreword

The last three decades have witnessed a revolution in the drug discovery and development process. Medicinal chemistry and in vitro screening that were once major bottlenecks in the process of identifying novel therapeutics have been dramatically accelerated through the incorporation of automation and the development of enabling technologies such as recombinant DNA and transfection technology. High-throughput screening (HTS), parallel synthesis, and combinatorial chemistry have facilitated the synthesis and biological evaluation of large numbers of potentially useful compounds. These activities, in turn, have generated vast amounts of data that can be analyzed to develop structure–activity relationships and structure–property relationships useful for the optimization of lead compounds. At the same time, new techniques, technological advances, and a greater understanding of the importance of pharmacokinetics, animal models, and safety studies have dramatically altered how new molecules are selected for clinical study. Design strategies of clinical trials, biomarkers, translational medicine, the regulatory landscape, intellectual property rights, and the business environment have also changed dramatically over the course of the last 30 years.

The complexities of the drug discovery and development process cannot be overstated, nor can the wide range of expertise required for the successful development of new, marketable therapeutics. In order to thrive in this every changing landscape, individuals interested in a career in the pharmaceutical industry or related fields must be more than simply experts in their chosen field of study. They must also have an understanding of the numerous, overlapping fields of their colleagues. *Basic Principles in Drug Discovery and Development* has captured the critical information on the disparate processes, technologies, and expertise required for modern drug discovery and development and presents it in a logical and concise manner for students, practicing scientists, and nonscientists with an interest in the pharmaceutical industry. Dr Benjamin E. Blass, an experienced educator and scientist with foundational knowledge in medicinal chemistry, drug design, biological targets, and over 20 years of experience in industrial and academic drug discovery and development, provides a comprehensive account of the many functions involved in drug discovery and development, from initial medicinal chemistry conceptualization and in vitro biological evaluation to clinical trials and beyond.

There are many aspects of this book that will help practicing scientists, graduate students, and future drug discovery researchers to develop a strong foundation in the concepts that govern the multidisciplinary process of drug discovery. Through this unique text, they will acquire an understanding of key aspects of drug discovery and development. The organization of the subject material was chosen to allow the readers to incrementally increase their knowledge in the wide range of disciplines required to identify new, marketable therapeutic agents. The book is thoroughly written and includes 13 chapters with over 300 figures and 900 references. Throughout the text, the reader will become familiar with more than 100 drugs and clinical candidates that exemplify important theories and practices.

Each chapter contains examples of drugs pertaining to the material in the chapter. The opening chapter provides an overview of drug discovery and development. This serves as the foundation for the following 10 chapters which describe the various functions involved in drug discovery and development. The early phases of drug discovery are described in detail through discussions of important topics such as target identification, target validation, lead identification, multidimensional lead optimization, pharmacokinetics, preclinical pharmacodynamics, and early toxicology. This is followed by discussions of preclinical activities, clinical trial design, biomarkers, and translational medicine. Each chapter builds on the previous chapters and this approach provides the readers with an integrated view of the various, multidisciplinary functions required for the drug discovery and development process.

Chapters 11 and 12 describe two important topics essential for running an effective pharmaceutical R&D business: organizational structure and patent protection. These chapters give the reader a true understanding of the organizational structure required for the successful management of research and development organizations and the importance of protecting intellectual property to ensure a good return on investment. Patent protection is the life blood of the pharmaceutical and biotech industries, and at the same time a source of innovation for new discoveries. Patents ensure the sharing of discoveries and innovations that might otherwise be kept as trade secrets. In the final chapter, case studies demonstrating the practical application of the concepts and principles described in the previous chapters are provided. These vignettes also describe important lesson learned in each case, some of which changed the way modern drug discovery research and development programs are executed.

Although there are numerous textbooks that discuss various aspects of the drug discovery and development process, none of them provides a comprehensive view of the process. *Basic Principles in Drug Discovery and Development* is unique in its comprehensive approach to this

complex endeavor. In writing this textbook, Dr Blass has provided an important new tool for the education of the next generation and a valuable resource for people with a vested interest in the identification and commercialization of novel medications.

Magid Abou-Gharbia, PhD, FRSC

Associate Dean for Research

Laura H. Carnell, Professor

Director Moulder Center for Drug Discovery Research

School of Pharmacy, Temple University

Philadelphia, PA, USA

Contents

Foreword

xiii

1. Drug Discovery and Development: An Overview of Modern Methods and Principles	1
Drug Discovery and Development from 20,000 Feet	8
Target Selection: The First Step Forward	11
Hit Identification: Finding a Starting Point	15
Identify a Clinical Candidate: Juggling the Properties	21
Questions	26
References	27
2. The Drug Discovery Process: From Ancient Times to the Present Day	35
The Age of Botanicals: Preindustrial Drug Discovery	36
Paul Ehrlich: The Father of Modern Drug Discovery	39
Milestones in Drug Discovery	41
Milestones in Animal Models: Breeding a Better Model	42
The Wistar Rat	42
Immunocompromised Mice	43
Transgenic Animal Models	44
Knockout Animal Models	45
Milestones in Molecular Science	47
X-ray Crystallography	48
Molecular Modeling and Computational Chemistry	50
High Throughput Technology: Chemical Synthesis and Screening Science	53
Milestones in Biotechnology	59
Recombinant DNA and Transfection Technology	60
Polymerase Chain Reaction (PCR) Technology	62
Monoclonal Antibody and Hybridoma Technology	64
The Rise of Biologics and Macromolecular Therapeutics	66
Societal and Governmental Impacts	67
The Pure Food and Drug Act of 1906	68
The Elixir of Sulfanilamide Disaster of 1937	69
The Thalidomide Story	71

Regulatory Milestones	73
Durham–Humphrey Amendment of 1951	74
Kefauver–Harris Amendment of 1962	74
Hatch–Waxman Act of 1984	75
Biologics Price Competition and Innovation Act of 2009	77
Future Developments in Drug Discovery	78
Questions	78
References	79
 3. Classical Targets in Drug Discovery	 87
Protein Structure	89
Enzymes	95
Inhibition of Enzymes	101
G-Protein-Coupled Receptors (GPCRs)	105
G-Protein-Dependent Signaling Pathways	109
cAMP Signaling	110
IP ₃ Signaling	111
Modulating GPCR Activity	112
Ion Channels	116
Gating Mechanisms	120
Ligand-Gated Channels	120
Voltage-Gated Channels	123
Other Gating Mechanisms	126
Membrane Transport Proteins (Transporters)	126
Emerging Targets	134
Questions	135
References	136
 4. <i>In vitro</i> Screening Systems	 143
The Language of Screening: Basic Terms	144
Concentration Response Curves and IC ₅₀ s	145
Dissociation Constants (K _d) and Inhibition Constants (K _i)	146
Efficacy versus Binding: EC ₅₀ s	148
Agonist, Partial Agonist, Antagonist, Allosteric Modulators, and Inverse Agonists	149
Agonists and Partial Agonists	150
Antagonists	151
Basal Activity and Inverse Agonists	151
Allosteric Modulation	151
Receptor Reserve	152
Streptavidin and Biotin	153
Biochemical versus Cellular Assays	154
Assay Systems and Methods of Detection	156
Radioligand Assay Systems	157
Scintillation Proximity Assay (SPA)	159
Enzyme-Linked Immunosorbent Assay (ELISA)	162
Fluorescence-Based Assay Systems	164

Fluorescence Polarization (FP)	165
Fluorescence Resonance Energy Transfer (FRET)	168
Time-Resolved Fluorescence Resonance Energy Transfer (TRFRET)	172
Amplified Luminescent Proximity Homogeneous Assay (AlphaScreen™)	175
Fluorescent Detection of Calcium Flux	177
Reporter Gene Assays	180
Chloramphenicol Acetyltransferase (CAT)	181
β -Lactamase Reporter Assays	182
Luciferase Reporter Assays	183
Kinetic Fluorescent Measurement Systems	184
Label-Free Assay Systems	185
Cellular Dielectric Spectroscopy	186
Optical Biosensors	187
Surface Plasmon Resonance Technology	190
Electrophysiological Patch Clamp	191
General Consideration for All Screening Methods	194
Questions	196
References	197
 5. Medicinal Chemistry	 203
Structure–Activity Relationships and Structure–Property Relationships	204
The Role of Chirality	209
Push and Pull in Structure–Activity Relationships	212
Quantitative Structure–Activity Relationships	213
The Pharmacophore	218
Developing an SAR Data Set	220
The Structure–Activity Relationship Cycle	231
Bioisosterism	232
Structure–Activity Relationship, Selectivity and Physicochemical Properties	236
“Druglike” Guidelines	237
Questions	239
References	240
 6. <i>In vitro</i> ADME and <i>In vivo</i> Pharmacokinetics	 245
Absorption	249
Solubility	250
Permeability	254
Distribution	262
Permeability	264
Transporters	266
Plasma Protein Binding	268
Elimination Pathways	270
Metabolism	271
Excretion	283
<i>In vitro</i> ADME Screening Methods	287

<i>In vivo</i> Pharmacokinetics	289
Volume of Distribution	291
Clearance	293
Half-life	294
Bioavailability	296
Species Selection	299
Questions	300
References	301
 7. Animal Models of Disease States	 307
Sources of Animal Models	309
Validity of Animal Models	312
Species Selection	313
Number of Animals	314
Exemplary Animal Models by Disease Category	314
Animal Models in Neuroscience	315
The Forced Swimming Test: A Model of Depression	315
The Elevated Plus Maze: A Measure of Anxiety	315
The Novel Object Recognition Test: A Model of Memory and Cognition	316
Contextual Fear Conditioning Model: A Model of Contextual Learning	317
The Morris Water Maze: A Model of Spatial Learning and Memory	318
Animal Models of Neurodegeneration	320
The SOD1-G93A Mouse of Amyotrophic Lateral Sclerosis	320
The MPTP Model of Parkinson's Disease	321
Animal Models of Cardiovascular Disease	323
Models of Hypertension	323
Models of Hyperlipidemia and High Cholesterol	325
Models of Atrial Fibrillation	327
Models of Heart Failure	329
Animal Models of Infectious Disease	332
Murine Thigh Infection Model	332
Murine Model of Systemic Infection	333
The Mouse Model of Influenza Virus Infection	334
Limitations of Animal Models of Infection	335
Animal Models of Oncology	335
Mouse Xenograft Tumor Model	336
Mouse Allograft Tumor Model	337
Genetically Engineered Mouse Models of Cancer	338
Questions	339
References	340
 8. Safety and Toxicology	 345
Sources of Toxicity	347
Acute versus Chronic Toxicity	353
Cytotoxicity	354

Carcinogenicity, Genotoxicity, and Mutagenicity	356
Drug–Drug Interactions	360
Cardiovascular Safety and Toxicology Studies	362
Central Nervous System Safety and Toxicology Studies	369
Immune System Mediated Safety Issues	371
Teratogenicity	373
<i>In vivo</i> Toxicity and Safety Studies	375
Questions	376
References	377
 9. Basics of Clinical Trials	 383
Before the Clinic	386
Drug Supply	386
Delivery Methods	389
Formulation	391
Investigational New Drug Application	395
Phase I Clinical Trials	397
Phase II Clinical Trials	399
Phase III Clinical Trials	402
Phase IV Clinical Trials	407
Adaptive Clinical Trial Design	409
Questions	410
References	410
 10. Translational Medicine and Biomarkers	 415
Definition of a Biomarker and Their Classification	418
Characteristics and Impact of Biomarkers	421
Biomarkers versus Surrogate End Points	423
Imaging Technologies	424
The Practical Application of Biomarkers	432
DPP-IV Inhibitors (Januvia®)	432
Physiological Measurements as Biomarkers: Orexin Antagonists	434
FDG PET Imaging Agent	436
The Neurokinin 1 (NK ₁) Receptor, Depression, and PET Imaging:	
The Aprepitant Story	438
Questions	440
References	440
 11. Organizational Considerations and Trends in the Pharmaceutical Industry	 447
Organizational Structures of Pharmaceutical Companies	448
Business Divisions Interactions	448
The Discovery Project Team Evolutionary Cycle	449
The Business Climate	452
Mergers and Acquisitions	453
Contract Research Organizations	457

Academic Drug Discovery	460
Funding Issues	465
Questions	467
References	468
12. Intellectual Property and Patents in Drug Discovery	471
Patentable Subject Matter	473
Inherent Properties and Patentability	477
Novelty and the Prior Art	479
Obviousness and the Prior Art	480
Inventorship	483
Assignment and Ownership	485
Classification of Patents and Patent Applications	486
Impact of Overlapping Patents	487
Patent Applications and Their Contents	488
Contents of a Patent Application	492
Questions	497
References	497
13. Case Studies in Drug Discovery	499
Tamiflu: From Mechanism of Action to Marketed Drug	499
Histone Deacetylase Inhibitors: Physicochemical Optimization via Structural Change	504
HIV Protease Inhibitors: Chemically Complex Miracle Drugs	506
Nitrofurantoin: A Surprisingly Successful Drug	511
Seldane® (Terfenadine) versus Allegra® (Fexofenadine): Metabolism Matters: Safety	513
Claritin® (Loratadine) versus Clarinex® (Desloratadine): Metabolism Matters: Pharmacokinetics	516
MPTP: Parkinson's Disease in a Bottle	517
Bupropion and Methylphenidate: Improving Performance via Formulation Changes	521
Selective Inhibition of Cyclooxygenase-2: The Impact of an Inadequate Written Description	524
Questions	526
References	526
Answers to Questions in Textbook by Chapter	531
Subject Index	557
Drug Index	571

Companion site for this book can be accessed at
<http://booksite.elsevier.com/9780124115088>