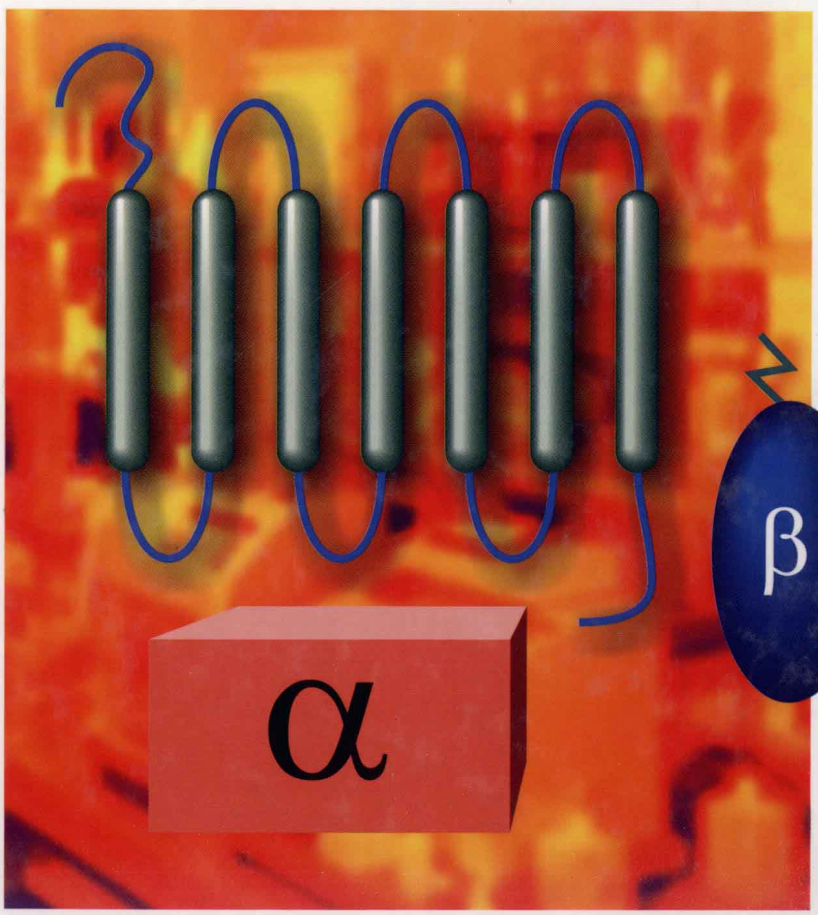
Methods and Principles in Medicinal Chemistry

Edited by Th. Dingermann,
D. Steinhilber, G. Folkers

WILEY-VCH

Molecular Biology in Medicinal Chemistry



Volume 21

Series Editors:
R. Mannhold,
H. Kubinyi,
G. Folkers

Molecular Biology in Medicinal Chemistry

Edited by Th. Dingermann, D. Steinhilber and G. Folkers

WILEY-VCH Verlag GmbH & Co. KGaA

Molecular Biology in Medicinal Chemistry

Edited by Th. Dingermann, D. Steinhilber and G. Folkers

Molecular Biology in Medicinal Chemistry. Edited by Th. Dingermann, D. Steinhilber, G. Folkers

ISBN: 3-527-30431-2

Methods and Principles in Medicinal Chemistry

Edited by R. Mannhold, H. Kubinyi, G. Folkers

Recently Published Volumes:

G. Molema, D. K. F. Meijer (eds.)

Drug Targeting

Vol. 12

2001, ISBN 3-527-29989-0

D. Smith, D. Walker, H. van de Waterbeemd

Pharmacokinetics and Metabolism in Drug Design

Vol. 13

2001, ISBN 3-527-30197-6

T. Lengauer (ed.)

Bioinformatics – From Genomes to Drugs

Vol. 14

2001, ISBN 3-527-29988-2

J. K. Seydel, M. Wiese

Drug-Membrane Interactions

Vol. 15

2002, ISBN 3-527-30427-4

O. Zerbe (ed.)

BioNMR in Drug Research

Vol. 16

2002, ISBN 3-527-30465-7

P. Carloni, F. Alber (eds.)

Quantum Medicinal Chemistry

Vol. 17

2003, ISBN 3-527-30456-8

H. van de Waterbeemd, H Lennernäs, P. Artursson (eds.)

Drug Bioavailability

Vol. 18

2003, ISBN 3-527-30438-X

H.-J. Böhm, G. Schneider (eds.)

Protein-Ligand Interactions

Vol. 19

2003, ISBN 3-527-30521-1

R. E. Babine, S. S. Abdel-Meguid (eds.)

Protein Crystallography in Drug Discovery

Vol. 20

2003, ISBN 3-527-30678-1

Series Editors:

Prof. Dr. Raimund Mannhold
Biomedical Research Center
Molecular Drug Research Group
Heinrich-Heine-Universität
Universitätsstraße 1
40225 Düsseldorf
Germany
raimund.mannhold@uni-duesseldorf.de

Prof. Dr. Hugo Kubinyi
BASF AG Ludwigshafen
c/o Donnersbergstraße 9
67256 Weisenheim am Sand
Germany
kubinyi@t-online.de

Prof. Dr. Gerd Folkers
Department of Applied Biosciences
ETH Zürich
Winterthurerstr. 190
8057 Zürich
Switzerland
folkers@pharma.anbi.ethz.ch

Volume Editors:

Prof. Dr. Theodor Dingermann
Institute of Pharmaceutical Biology
Johann Wolfgang Goethe-University
Marie-Curie-Str. 9
60439 Frankfurt
Germany
dingermann@em.uni-frankfurt.de

Prof. Dr. Dieter Steinhilber
Institute of Pharmaceutical Chemistry
Johann Wolfgang Goethe-University
Marie-Curie-Str. 9
60439 Frankfurt
Germany
steinhilber@em.uni-frankfurt.de

Prof. Dr. Gerd Folkers
Department of Applied Biosciences
ETH Zürich
Winterthurerstr. 190
8057 Zürich
Switzerland
folkers@pharma.anbi.ethz.ch

Library of Congress Card No. applied for
British Library Cataloguing-in-Publication Data: A catalogue record for this book is available from the British Library
Die Deutsche Bibliothek – CIP Cataloguing-in-Publication-Data: A catalogue record for this publication is available from Die Deutsche Bibliothek

Printed in the Federal Republic of Germany.
Printed on acid-free paper

Typesetting: Asco Typesetters, Hong Kong
Printing: betz-druck gmbh, Darmstadt
Bookbinding: Litges & Dopf Buchbinderei GmbH, Heppenheim

1st Edition 2004
1st Reprint 2004

ISBN 3-527-30431-2

Preface

Why address molecular biology and related technologies in a series named "Methods and Priciples in Medicinal Chemistry"? It was the advent of the silicon chip and the detection of DNA processing enzymes that jointly started an evolutionary track in the 1970s which boosted the whole variety of methodologies in what is today known as the life sciences. Also, the classical field of medicinal chemistry has been augmented and today comprises a huge range of techniques and methodologies from QSAR and structure-based design to the recently developed "high-throughput" synthesis and screening. A paradigmatic change in the 1990s gave rise to a focus on the molecular level of drug action and hence demanded the development of appropriate biological assay technology. This is the point where the present book starts.

In the first part, molecular targets are dealt with, going deep into cellular assay technologies. Cell-based assays imply not only the "simple" detection of one cellular product, but the tracking of a variety of metabolic processes, finally resulting in a multidimensional phenotypic characterization of cellular behavior. In a hierarchical step, the second chapter introduces the "gene knock-out" models, a technique that allows to "design" a disease model within a complex organism to generate a more relevant analytical tool for medicinal chemistry. The subsequent chapter deals with a fascinating readout technology for molecular assays, the so-called reporter genes.

The recent elucidation of the human genome has provided another boost to the whole field. Suddenly, a huge amount of targets was available for study. The question, however, which still remained was: What does the target do within the cellular biochemistry and how is it controlled? Those are the questions tackled in chapter 4, which deals with orphan receptors of the GPCR type and shows the challenges and the opportunities for finding new ligands with hitherto unknown biological activity.

The second part of the book is devoted to synthesis. Two important fields can benefit tremendously from molecular biology and its techniques: Stereoselective synthesis of natural compounds and of their mimics, and synthesis of DNA-derived drugs or protein drugs. The first chapter within this section gives a comprehensive overview about the use of enzymes in stereoselective synthesis, emphasizing recombinant technologies, which greatly enhance the selection of

working tools. The fascinating field of nucleic acid drugs, the design and synthesis, their mimics and their mechanisms of action are the topics of chapter 6.

The third part deals with questions of analysis. Invaluable contributions have come from use of proteins for enantioseparation and affinity chromatography. The use of NMR and associated techniques for structure elucidation is another analytical topic to read about in this section.

Kinetics, metabolism, toxicology, and the very rapidly growing fields of pharmacogenomics and toxicogenomics form the contents of the final part of this book which is unique in its presentation of today's entanglement of the biosciences with medicinal chemistry.

The editors are indebted to the volume editors and the authors, whose work and motivation adds an highly important and fascinating facet to the series and gratefully acknowledge the ongoing support from Frank Weinreich, Wiley-VCH, during the whole project.

September 2003

Raimund Mannhold, Düsseldorf
Hugo Kubinyi, Weisenheim am Sand
Gerd Folkers, Zürich

Foreword

Where to start and where to end was the initial question when we selected the subjects which should be included into a volume dealing with the application of molecular biology in medicinal chemistry. The enormous progress in molecular biology during the last decades has led to the development of many methods with impact on drug discovery and drug development as well.

Modern target identification and validation on the one hand, and drug development and characterization on the other hand require an overlapping spectrum of methods and assays which goes far beyond the classical methodological repertoire of medicinal chemistry. The detailed molecular characterization of interactions of drugs with their targets is as important and demanding as detailed knowledge on drug interactions with the entire physiological system. Concepts and assays are available that provide us with information on drug transport, metabolism and stability. But we also can determine and predict how a system will react upon the application of a drug, even if this drug is considered to act very specifically at a certain target. Methods and concepts of modern pharmacogenomics and toxicogenomics have clearly demonstrated this.

Considering all this we finally decided to organize this volume in four parts: (I) Molecular Targets, (II) Synthesis, (III) Analysis and finally (IV) Kinetics, Metabolism and Toxicology.

The first chapter in part I sets the biological stage by providing an up-to-date description of available "Cellular Assays" and their use and impact on "Drug Discovery". Assays for membrane proteins and fast cellular responses, assays for gene and protein expression profiling in high-throughput formats, spatio-temporal assays and subpopulation analysis as well as phenotypic assays are introduced.

More complex systems are addressed in the second chapter of part I, which deals with gene knockout mice and techniques available for the generation of such animals.

G-protein coupled receptors are addressed in the third and fourth chapter. In the first of these two chapters, the focus lies on the characterization of G-protein coupled receptors and the application of reporter genes as read out systems, while the subsequent chapter, as a reference to the postgenomic era, discusses strategies for the identification of ligands for orphan G-protein coupled receptors.

Part II of the volume covers several aspects of drug synthesis. A classical overlap

between organic synthesis and biotechnology is the stereoselective synthesis of drugs with the help of recombinant enzymes. The first chapter in this part gives an overview on this topic and provides many examples, underscoring the impact of such strategies.

Nucleic acid drugs eventually will come of age. Their attractiveness as potentially very specific ligands was always in conflict with numerous pharmacokinetic problems. However, various concepts for stabilizing these molecules, the fascinating potential of RNAi and the first approved drugs were strong reminders of these molecules, e.g. as manipulators of cellular signaling. The chapter by Engels and Parsch touches many aspects, including synthesis and application of this type of compounds, including the RNAi technology.

Part III of the book focuses on analytical aspects. Enantioseparation of chiral drugs and affinity chromatography are extremely important tools in drug development and comprehensive reviews on these topics are presented in chapters 7 and 8.

Analytical methods related to structural biology are included in a series of three articles. Two of these papers deal with NMR technologies that have strongly developed during the last decades. This led to the establishment of NMR as an relevant tool for the determination also of macromolecular structures and for the detection and characterization of ligand-target interactions. Chapters 9 and 10 summarize the application of NMR in drug discovery and describe techniques for ^{13}C- and ^{15}N-isotopic labeling of proteins.

Rational drug design depends on exact structure knowledge, which is still best provided by X-ray crystallography. Despite of extreme methodological improvements in this field, structures of membrane receptors, which represent the most important drug targets, are not available. A strong move towards solving this problem might be the use of antibody fragments as crystallization enhancers. Details of this exciting new technique are described in the final chapter of part III.

Pharmacogenomics and toxicogenomics are new fields with considerable impact on future drug development. The last section of the book covers these hot topics, which will eventually initiate the change from the "one size fits all" concept to the "right drug, right size, right person" concept.

The editors would like to gratefully acknowledge the contributions of all authors. They also thank Dr. Frank Weinreich and Wiley-VCH for a steady support during the ongoing project.

September 2003

Theo Dingermann, Frankfurt
Dieter Steinhilber, Frankfurt
Gerd Folkers, Zürich

Contributors

Dr. Hugo Albrecht
Discovery Partners Interational AG
Gewerbestrasse 16
4123 Allschwil
Switzerland

Dr. Remko A. Bakker
Leiden/Amsterdam Center for Drug Research
Department of Medicinal Chemistry
Vrije Universiteit Amsterdam
De Boelelaan 1083
1081 HV Amsterdam
The Netherlands

Prof. Dr. Annette G. Beck-Sickinger
Institute of Biochemistry
University of Leipzig
Talstrasse 33
04103 Leipzig
Germany

Dr. Frank Bernhard
Institute of Biophysical Chemistry
Johann Wolfgang Goethe-University
Marie-Curie-Strasse 9
60439 Frankfurt
Germany

Dr. Daniela Brodbeck-Hummel
Discovery Partners Interational AG
Gewerbestrasse 16
4123 Allschwil
Switzerland

Prof. Dr. Ingolf Cascorbi
Institute of Pharmacology
Ernst-Moritz-Arndt-University Greifswald
Friedrich-Loeffler-Strasse 23D
17487 Greifswald
Germany

Prof. Dr. Bezhan Chankvetadze
Institute of Pharmaceutical and Medicinal Chemistry
University of Münster
Hittorfstrasse 58–62
48149 Münster
Germany

Dr. Michaela C. Dinger
Institute of Biochemistry
University of Leipzig
Talstrasse 33
04103 Leipzig
Germany

Prof. Dr. Theo Dingermann
Institute of Pharmaceutical Biology
Johann Wolfgang Goethe-University
Marie-Curie-Strasse 9
60439 Frankfurt
Germany

Prof. Dr. Joachim Engels
Institut of Organic Chemistry and Chemical Biology
Johann Wolfgang Goethe-University
Marie-Curie-Strasse 11
60439 Frankfurt
Germany

Dr. Christina Fischer
Institute of Biophysical Chemistry
Johann Wolfgang Goethe-University
Marie-Curie-Str. 9
60439 Frankfurt
Germany

Prof. Dr. Gerd Folkers
Institute of Pharmaceutical Sciences
ETH Zürich
Wintherthurerstrasse 190

8057 Zürich
Switzerland

Dr. Ulrich L. Günther
Institute of Biophysical Chemistry
Johann Wolfgang Goethe-University
Marie-Curie-Str. 9
60439 Frankfurt
Germany

Dr. Wilbert H. M. Heijne
TNO Food and Nutrition Research
Department of Explanatory Toxicology
Utrechtseweg 48
P.O. Box 360
3600 AJ Zeist
The Netherlands

Dr. Michael Hoever
Discovery Partners Interational AG
Gewerbestrasse 16
4123 Allschwil
Switzerland

Dr. Carola Hunte
Max-Planck-Institute of Biophysics
Department of Molecular Membrane Biology
Marie-Curie-Strasse 13–15
60439 Frankfurt
Germany

Dr. Kurt Kesseler
Industriepark Höchst
Building H 823
65926 Frankfurt am Main
Germany

Dr. Christian Klammt
Institute of Biophysical Chemistry
Johann Wolfgang Goethe-University
Marie-Curie-Strasse 9
60439 Frankfurt
Germany

Prof. Dr. Heyo K. Kroemer
Institute of Pharmacology
Ernst-Moritz-Arndt-University Greifswald
Friedrich-Loeffler-Strasse 23D
17487 Greifswald
Germany

Prof. Dr. Rob Leurs
Leiden/Amsterdam Center for Drug Research
Department of Medicinal Chemistry
Vrije Universiteit Amsterdam
De Boelelaan 1083
1081 HV Amsterdam
The Netherlands

Dr. Andreas Liese
Institute of Biotechnology 2
Research Center Jülich GmbH
P.O. Box 1913
52425 Jülich
Germany

Dr. Cornelia Münke
Max-Planck-Institute of Biophysics
Department of Molecular Membrane Biology
Marie-Curie-Strasse 13–15
60439 Frankfurt
Germany

Dr. Beatrice Nickel
Discovery Partners Interational AG
Gewerbestrasse 16
4123 Allschwil
Switzerland

Dr. Ben van Ommen
TNO Food and Nutrition Research
Department of Explanatory Toxicology
Utrechtseweg 48
P.O. Box 360
3600 AJ Zeist
The Netherlands

Dr. Jörg Parsch
Beilstein Chemiedaten und Software GmbH
Trakehner Strasse 7–9
60487 Frankfurt
Germany

Dr. Nagaraj Rao
Rane Rao Reshamia Laboratories Pvt. Ltd.
Plot 80, Sector 23
Turbhe
Navi Mumbai 400705
India

Dr. Urs Regenass
Discovery Partners Interational AG
Gewerbestrasse 16
4123 Allschwil
Switzerland

Prof. Dr. Heinz Rüterjans
Institute of Biophysical Chemistry
Johann Wolfgang Goethe-University
Marie-Curie-Strasse 9
60439 Frankfurt
Germany

Prof. Dr. Peter Ruth
Institute of Pharmacy
University of Tübingen
Auf der Morgenstelle 8

72076 Tübingen
Germany

Dr. Matthias Sausbier
Institute of Pharmacy
University of Tübingen
Auf der Morgenstelle 8
72076 Tübingen
Germany

Prof. Dr. Gerhard K. E. Scriba
Institute of Pharmacy
Friedrich-Schiller-University Jena
Philosophenweg 14
07743 Jena
Germany

Prof. Dr. Dieter Steinhilber
Institute of Pharmaceutical Chemistry
Johann Wolfgang Goethe-University
Marie-Curie-Strasse 9
60439 Frankfurt
Germany

Dr. Rob H. Stierum
TNO Food and Nutrition Research
Department of Explanatory Toxicology
Utrechtseweg 48
P.O. Box 360
3600 AJ Zeist
The Netherlands

Contents