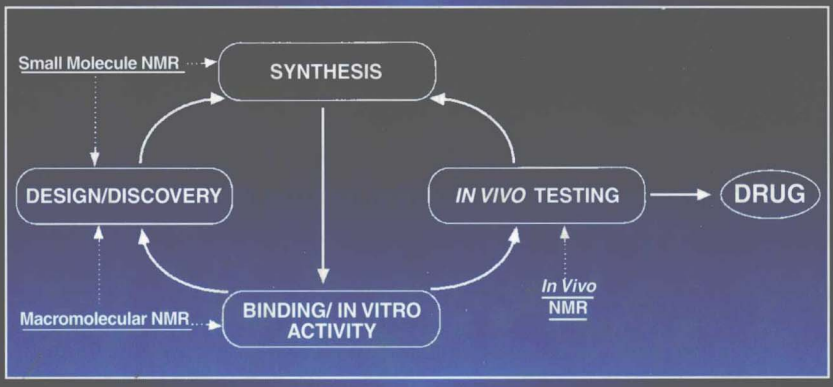
CRC Series in ANALYTICAL BIOTECHNOLOGY

NMR IN DRUG DESIGN



Edited by

David James Craik

NMR IN DRUG DESIGN

Edited by

David James Craik, Ph.D.

Professor of Biomolecular Structure

Centre for Drug Design and Development

University of Queensland

Brisbane

Queensland, Australia

CRC Press

Boca Raton New York London Tokyo

Library of Congress Cataloging-in-Publication Data

Craik, David James.
NMR in drug design / David James Craik
p. cm. -- (Analytical biotechnology)
Includes bibliographical references and index.
ISBN 0-8493-7824-9 (alk. paper)
1. Drugs--Design. 2. Nuclear magnetic resonance spectroscopy.
I. Title. II. Series: CRC series in analytical biotechnology.
RS420.C73 1995
615'.1901--dc20 95-34213
CIP

International Standard Book Number 0-8493-7824-9
Library of Congress Card Number 95-34213
Printed in the United States of America 1 2 3 4 5 6 7 8 9 0
Printed on acid-free paper

FOREWORD

NMR spectroscopy is an extremely useful tool in drug research. In addition to its utility for determining the primary structures of bioactive natural products and chemically synthesized compounds, NMR has the potential to aid in the design of new pharmaceutical agents. This has become possible due to rapid advances in several areas of research. Proteins that function as drug targets can now be rapidly cloned, expressed, isotopically labeled, and purified in large quantities for NMR studies. Heteronuclear multi-dimensional NMR methods have been developed to assign and determine the three-dimensional structures of biomacromolecules and molecular complexes in a relatively short period of time (2 months for a protein of ~100 residues). In addition, computational tools have been developed and refined to take advantage of the structural information that can be obtained from the NMR experiments.

The type of structural information that can be obtained from NMR includes: the conformation of protein-bound ligands, the portion of the ligand that interacts with the biomacromolecule, the protonation state of the ligand, and the location and structure of the ligand-binding site. All of this information is potentially useful in the drug design process. However, some challenges and questions still remain. Is the resolution of the NMR-derived structures sufficient to be used in interactive structure-based design cycles — an approach that has proved to be successful when based on high resolution X-ray structures? Are the computational tools that ignore entropic effects and possible conformational changes in the protein and the ligand good enough for structure-based drug design? Which of the NMR-derived structures in the ensemble does one use in the design of new analogs?

As more experience is gained in the utilization of NMR-derived structures in the drug design process, the strengths and limitations of the method will be revealed. It has only recently become possible to obtain suitable

quantities of drug receptors and to rapidly determine their three-dimensional structures. Thus, the use of NMR in this regard is still in its infancy, but the future holds great promise for the application of NMR in drug design.

Stephen W. Fesik
Abbott Laboratories
Abbott Park, Illinois

INTRODUCTION

Nuclear Magnetic Resonance spectroscopy is an exquisitely powerful technique for providing the kind of information that is required by researchers involved in the design and development of new drugs. There is a large amount of literature available in the separate fields of NMR spectroscopy and drug design, and there are several specialist journals devoted to each of these fields. However, there is no existing publication which draws the two fields together as is attempted in the following chapters. This book should be useful both for researchers involved in drug design and for NMR spectroscopists interested in biological applications. In particular, it will allow medicinal chemists and pharmacologists working in drug design to appreciate the scope and limitations of NMR spectroscopy and will assist NMR spectroscopists to understand the problems inherent in drug design, thereby stimulating them to design novel experiments to assist in this important area.

The fields of NMR and drug design are both in phases of rapid expansion. In the case of NMR spectroscopy, there is currently great emphasis on the development of new pulse sequences and methodology for extracting an increasingly detailed level of information from the sample under study. This development is particularly intense in applications to the study of macromolecules and their interactions with ligands. Drug design is expanding on several frontiers, not the least of which is an increasing trend toward computational approaches to drug design. One reason for this relates to the increasing power and decreasing cost of computational and graphics hardware, combined with a significant expansion in available software. The high cost of the synthesis of large numbers of drug analogs for testing can be significantly reduced if computational procedures can provide some initial screening. An issue that is addressed in this book is that while computational approaches are extremely useful guides for the synthesis of lead compounds, it is important to have an **experimental** means of confirming that the synthesized compounds bind to their macromolecular targets in the way in which they were designed to bind. This information is an essential prerequisite to further refinement of lead compounds. X-ray crystallography has traditionally been an important tool for providing this information in receptor-based drug design and is characterized

by the high degree of accuracy with which the complex between a drug and its target macromolecule may be determined. It has only been over the last few years that NMR spectroscopy has been able to approach the sort of atomic resolution seen in X-ray crystallography, and knowledge in the field is still evolving rapidly.

From the comments above it is clear that methods which determine not just the structure of a drug, or its receptor, but the nature of their interaction, will be important in the drug design process. NMR offers the opportunity to derive this information. It is hoped that the current volume will, by describing advances so far, stimulate further developments in the field.

This book is divided into twelve chapters. The first provides an overview of the fields of drug design and drug discovery, and traces the history of these alternative starting points for the development of new pharmaceutical compounds. This historical overview is used to set the scene for a discussion of the current state-of-the-art in drug development and to indicate where it may next be heading. It is interesting to see that the two approaches to drug development — discovery (based on traditional medicine, chance observations, or random screening), and design (based on theories of selective toxicity, molecular optimization, and knowledge of biomolecular structures) — are converging and that most major pharmaceutical companies are committed to a combination of both approaches. Chapter 1 highlights the areas in which NMR will play an increasingly important role in the future.

Chapter 2 identifies the contributions that NMR has made in the past to the pharmaceutical sciences, with particular reference to drug development, and gives a brief introduction to the capabilities of modern NMR spectroscopy. Over the last decade NMR has undergone an astounding increase in the capabilities of both instrumentation and methodology, and these have dramatically expanded the potential applications in drug design. Ten years ago the use of NMR to determine protein structures was in its embryonic stages, but today it competes with, and complements, X-ray crystallography as a premier technique for structure determination of medium-sized proteins.

One of the aims of this book is to assist drug researchers who may have little knowledge of NMR to appreciate its potential applications in their field. Drug design and development is a multidisciplinary field that involves chemists, biochemists, pharmacologists, and medically oriented researchers. It is unusual for those at one end of this spectrum of expertise to have been fully exposed to techniques at the other end of the spectrum. In planning this book, it was therefore important to include a chapter in which some of the principles of NMR and some of the important measurable NMR parameters could be described at a level suitable for a non-expert. Chapter 3 achieves this aim and provides an extremely thorough

account of the parameters and methods of modern NMR spectroscopy that are relevant in drug design. It provides a basis for the understanding of the many applications and examples described in the following chapters. The chapter is equally useful for NMR experts, as many topics are dealt with at a more advanced level. In particular, the role of chemical exchange is clearly fundamental in drug action, as it is central to receptor binding. The third chapter covers this important field with an attention to detail that makes it valuable as a reference chapter for NMR researchers whose focus may be in fields other than drug design, but who deal with chemical exchange processes in their applications.

Chapter 4 describes the state-of-the-art in protein structure determination by NMR. Proteins of increasing size are being studied in increasing numbers as instrumentation improves and as new NMR methods are developed to overcome some of the limitations present in 'traditional' approaches to structure determination (if it is appropriate to use the word traditional for a field that is essentially only a decade old!). The advantages of labelling proteins with ^{13}C and/or ^{15}N isotopes are described, and a section on the methods for doing this is included. As with Chapter 3, this chapter provides a valuable reference source for both the expert and nonexpert in the field.

With the scene set by the introductory chapters, and the relevant methods described in Chapters 3 and 4, the remaining chapters focus on applications in drug design and development. Chapter 5 concentrates on drugs themselves. While to some extent the 'glamour' applications of NMR over the last few years have been associated with macromolecules, including many drug targets, it is important to remember that NMR has contributed very substantially to our understanding of molecular structure and conformations of small molecules. The average molecular weight of the most commonly used drugs today is still, after all, only approximately 300 Da. Applications involving the identification and characterization of such species will continue to be of paramount importance.

Peptides, or analogues derived from them, have been widely touted as the drugs of the future. In Chapter 6 the methods that are particularly valuable for the study of these fascinating and potent molecules are described. The possibilities for conformational variability in even small peptides are endless, and approaches to limiting this flexibility are described. NMR plays a crucial role in establishing the nature and effectiveness of many of these conformational constraints. The potential to use conformations derived by NMR for the design of potent peptide mimics is outstanding.

It is important to emphasize that NMR plays a very practical role in drug development and is not just an esoteric technique confined to academic questions of molecular structure. It is therefore particularly gratifying to be able to include several chapters from researchers actively

working in the pharmaceutical industry. Chapter 7 is one of these and is packed with examples showing how NMR has contributed vital information in drug design programs. As in the previous chapter, the importance of recognizing differences between solution and receptor-bound conformations is emphasized.

Dihydrofolate reductase is a key target for a number of drugs in clinical use and it provides a classic example of the extent of information that can be obtained by NMR. This enzyme is one of the most thoroughly studied by NMR and the author of Chapter 8 has been at the forefront of this work. This chapter illustrates the incredible level of detail about ionization states, functional group interactions, and binding modes that is provided by carefully designed NMR studies. It also illustrates the value of NMR in identifying multiple binding modes, a factor that complicates drug design if applied in a naive sense. The fact that similar ligands can sometimes bind in quite different orientations shows just how important it is to continue to develop methods that define binding modes with very high accuracy.

Chapter 9 is another contribution from an active NMR research group within the pharmaceutical industry and provides an insight into drug binding to the important target protein, calmodulin. This chapter is an up-to-date account of work in-progress on the binding of calmidazolium, a potent inhibitor of calmodulin function. It emphasizes the central importance of ligand-macromolecule interactions in drug design. Once again the value of using isotope labels is illustrated.

It is increasingly being recognized that it is not only the structure and geometry of molecules that determine their binding but also their dynamics. Both drugs and their receptors may undergo conformational changes on binding, and these changes may have an integral effect on the resulting response. In Chapter 10, NMR approaches to the study of molecular flexibility of an important drug target enzyme, HIV-1 protease, are described. Information obtained from such studies significantly extends our understanding of the mechanism of action of this enzyme.

DNA is another important target for drugs and the final two chapters describe NMR approaches that have been applied in studies of ligand binding to this macromolecule. In Chapter 11, the focus is on compounds that intercalate between the base pairs of DNA. This chapter includes a discussion on the role of DNA as a drug target. In Chapter 12, the minor groove of DNA is the site of interest and studies involving a range of drugs and other ligands are described.

Together these twelve chapters provide an up-to-date account of the contribution of NMR to drug design. Used in combination with other methods, including traditional medicinal chemistry, X-ray crystallography, and computational approaches, NMR promises to increase the success rate and reduce the lead time in drug development.

In closing, I would like to sincerely thank all of the authors for their contributions to this book. Their care in the preparation of their chapters was greatly appreciated and made the editorial process far easier than it might otherwise have been. It has been a pleasure to read the various chapters and to correspond with the authors.

Finally, I would like to thank my colleagues John Gehrmann, Justine Hill, Kathy Nielsen, and Martin Scanlon for their invaluable assistance in proofreading the various manuscripts. Special thanks go to John and Justine for their extra help in the production process, their help with diagrams, and for the countless trips to the photocopier. I am also very grateful to Jacqui King for her invaluable assistance with typing, correspondence, and other editorial matters. I also thank my wife, Robyn, for additional editorial assistance and for her patience with me during the production of this book.

David J. Craik

THE EDITOR

David J. Craik, Ph.D., is Professor of Biomolecular Structure at the Centre for Drug Design and Development, University of Queensland, Brisbane, Australia. He received his B.Sc. (Hons) degree from La Trobe University in 1977 and completed a Ph.D. on applications of NMR spectroscopy in organic chemistry at La Trobe in 1980. Following two years of postdoctoral work in the United States at Florida State and Syracuse Universities, he returned to Australia to a Lectureship, and in 1986 a Senior Lectureship, in Pharmaceutical Chemistry at the Victorian College of Pharmacy in Melbourne. In 1988 he was appointed Professor of Medicinal Chemistry and Dean of the School of Pharmaceutical Chemistry at Victorian College of Pharmacy, Monash University, a position he held until moving to the University of Queensland in 1995.

Professor Craik is a Fellow of the Royal Australian Chemical Institute, a member of the American Chemical Society, and is a Professorial Associate at the University of Melbourne, Australia and the University of Kansas in Lawrence, Kansas. He is Associate Editor of the journal, *Current Medicinal Chemistry.*

His major research interests are in applications of NMR to drug design and development. Current research activities focus on the use of NMR to study macromolecule-ligand interactions and on the study of biologically important peptides and proteins. His contribution to medicinal chemistry applications of NMR was recognized in 1993 by the award of the Adrien Albert Lectureship of the Division of Medicinal and Agricultural Chemistry, Royal Australian Chemical Institute. Professor Craik is the author of more than 120 research publications and a dozen reviews and chapters in the fields of NMR and medicinal chemistry.

CONTRIBUTORS

Peter R. Andrews, Ph.D.
Centre for Drug Design and Development
University of Queensland
Brisbane, Australia

Chong-Hwan Chang, Ph.D.
DuPont Merck Pharmaceutical Company
DuPont Experimental Station
Wilmington, Delaware

Robert M. Cooke, Ph.D.
Department of Biomolecular Structure
Glaxo Wellcome
Medicines Research Centre
Stevenage, England

David J. Craik, Ph.D.
Centre for Drug Design and Development
University of Queensland
Brisbane, Australia

Andrew J. Edwards
Analytical Sciences Department
SmithKline Beecham Pharmaceuticals
The Frythe, Welwyn, England

James Feeney, Ph.D.
Division of Molecular Structure
National Institute for Medical Research
The Ridgeway, Mill Hill,
London, England

J. T. Gerig, Ph.D.
Department of Chemistry
University of California
Santa Barbara, California

C. Nicholas Hodge, Ph.D.
DuPont Merck Pharmaceutical Company
DuPont Experimental Station
Wilmington, Delaware

Jerzy W. Jaroszewski, Ph.D.
Department of Medicinal Chemistry
Royal Danish School of Pharmacy
Copenhagen, Denmark

Horst Kessler, Ph.D.
Institute of Organic Chemistry and Biochemistry
Technical University Munich
Garshing, Germany

Glenn F. King, Ph.D.
Department of Biochemistry
University of Sydney
Sydney, NSW, Australia

Robert Konat, M.Sc.
Institute of Organic Chemistry and Biochemistry
Technical University Munich
Garshing, Germany

Joel Mackay, Ph.D.
Department of Biochemistry
University of Sydney
Sydney, NSW, Australia

Linda K. Nicholson, Ph.D.
Section of Biochemistry, Molecular and Cell Biology
Cornell University
Ithaca, New York

Spiro Pavlopoulos
Department of Medicinal Chemistry
Victorian College of Pharmacy
Monash University
Parkville, Victoria, Australia

David G. Reid, Ph.D.
Analytical Sciences Department
SmithKline Beecham Pharmaceuticals
The Frythe, Welwyn, England

Mark S. Searle, Ph.D.
Department of Chemistry
University of Nottingham
Nottingham, England

Wolfgang Schmitt, M.Sc.
Institute of Organic Chemistry and Biochemistry
Technical University Munich
Garshing, Germany

Patricia J. Sweeney, Ph.D.
Cantab Pharmaceutical Research Ltd.
Cambridge, England

Geoffrey Wickham, Ph.D.
Department of Chemistry
University of Wollongong
Wollongong, NSW, Australia

To Robyn, Andrew, Peter, and Jennifer

CONTENTS

CHAPTER 1

DRUG DESIGN AND DISCOVERY: WHERE NEXT?

Peter R. Andrews

CONTENTS

0-8493-7824-9/96/$0.00+$.50