Fragment-based Approaches in Drug Discovery

Edited by
Wolfgang Jahnke and Daniel A. Erlanson

WILEY-VCH Verlag GmbH & Co. KGaA

Fragment-based Approaches in Drug Discovery

Edited by
Wolfgang Jahnke and
Daniel A. Erlanson

Methods and Principles in Medicinal Chemistry

Previous Volumes of this Series:

R. Seifert, T. Wieland (eds.)

G-Protein Coupled Receptors as Drug Targets

Vol. 24

2005, ISBN 3-527-30819-9

O. Kappe, A. Stadler

Microwaves in Organic and Medicinal Chemistry

Vol. 25

2005, ISBN 3-527-31210-2

W. Bannwarth, B. Hinzen (eds.)

Combinatorial Chemistry

Vol. 26, 2nd Ed.

2005, ISBN 3-527-30693-5

G. Cruciani (ed.)

Molecular Interaction Fields

Vol. 27

2005, ISBN 3-527-31087-8

M. Hamacher, K. Marcus, K. Stühler, A. van Hall, B. Warscheid, H. E. Meyer (eds.)

Proteomics in Drug Design

Vol. 28

2005, ISBN 3-527-31226-9

D. Triggle, M. Gopalakrishnan, D. Rampe, W. Zheng (eds.)

Voltage-Gated Ion Channels as Drug Targets

Vol. 29

2006, ISBN 3-527-31258-7

D. Rognan (ed.)

Ligand Design for G Protein-coupled Receptors

Vol. 30

2006, ISBN 3-527-31284-6

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

Pharmacokinetics and Metabolism in Drug Research

Vol. 31, 2nd Ed.

2006, ISBN 3-527-31368-0

T. Langer, R. D. Hofmann (eds.)

Pharmacophores and Pharmacophore Searches

Vol. 32

2006, ISBN 3-527-31250-1

E.R. Francotte, W. Lindner (eds.)

Chirality in Drug Research

Vol. 33

2006, ISBN 3-527-31076-2

Fragment-based Approaches in Drug Discovery

Edited by
Wolfgang Jahnke and Daniel A. Erlanson

WILEY-VCH Verlag GmbH & Co. KGaA

Series Editors

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

Prof. Dr. Hugo Kubinyi
Donnersbergstrasse 9
67256 Weisenheim am Sand
Germany
kubinyi@t-online.de

Prof. Dr. Gerd Folkers
Collegium Helveticum
STW/ETH Zürich
8092 Zürich
Switzerland
folkers@collegium.ethz.ch

Volume Editors

Dr. Wolfgang Jahnke
Novartis Institutes for Biomedical Research
Novartis Pharma AG
Lichtstrasse
4002 Basel
Switzerland
wolfgang.jahnke@novartis.com

Dr. Daniel A. Erlanson
Sunesis Pharmaceuticals, Inc.
341 Oyster Point Boulevard
South San Francisco, CA 94080
USA
erlanson@sunesis.com

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

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at http://dnb.ddb.de.

Cover SCHULZ Grafik Design, Fußgönheim
Composition ProSatz Unger, Weinheim
Printing Strauss GmbH, Mörlenbach
Bookbinding Litges & Dopf GmbH, Heppenheim

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

ISBN-13: 978-3-527-31291-7
ISBN-10: 3-527-31291-9

Contents

Preface

"The whole is more than the sum of its parts" is a phrase that is attributed to the Greek philosopher Aristotle (384–322 BC), who in his book Metaphysica compared a syllable with its individual letters. Applied to medicinal chemistry, it means that an active molecule is more than its parts and pieces. In this respect, we need not step down to the level of individual atoms, it is just enough to consider larger fragments of a protein ligand. More than 30 years ago, Green dissected the avidin ligand biotin into a methyl-substituted imidazolinone, hexanoic acid, and a sulfur atom. The binding affinities of the two organic fragments were several decades lower than the affinity of the original ligand or of desthio-biotin. This was a clear indication that the proper combination of fragments may lead to high-affinity ligands. However, the result corresponded to expectation and seemingly nobody concluded to go the other way, i.e. to combine fragments to a high-affinity ligand. Later, Page and Jencks formulated the "anchor principle": if two molecules A and B, both interacting with different pockets of the binding site of a protein, are combined to A–B, one molecule may be considered as a substituent of the other one. The entropy loss from freezing translational and rotational degrees of freedom can be attributed to one of the molecules; the other molecule contributes to affinity with its "intrinsic" free energy of binding, without an unfavorable entropy term. In this manner, a higher affinity of A-B is observed than expected from the affinities of the original molecules A and B. Of course, both fragments have to be combined in a relaxed manner and the final molecule has to fit the binding site without steric or other constraints.

In the following years, several authors confirmed the observation that the affinity of a ligand is more than the "sum of its fragments". Surprisingly, only ten years ago this principle was used for a systematic design of protein ligands from fragments by the "SAR by NMR" method, developed by Fesik and his group at Abbott Laboratories. Several other techniques followed, using protein crystallography, NMR, MS, cysteine tethering, or the dynamic assembly of ligands, to mention only some approaches. Within a short time, fragment-based design became a hot topic in drug discovery and in experimental techniques, as well as in cheminformatics and virtual screening. In addition to drug- and lead-likeness, desirable properties of fragment libraries were also defined and libraries for screening and

docking were generated, using these property definitions. It is clear that the combination of a limited number of fragments generates a multitude of different combinations, making this approach as attractive as combinatorial chemistry - without the need for producing millions of molecules.

This book is the very first to provide a comprehensive overview on this fascinating area, which opens a new perspective for the rational design of potential drugs. It is hoped that its content stimulates further research and strengthens the role of structure-based design in drug discovery.

We would like to express our gratitude to the editors Wolfgang Jahnke and Dan Erlanson, who assembled this book in short time, despite their hard work and responsibilities in their companies. We are also very grateful to all chapter authors, who accepted the invitation to contribute and to deliver their manuscripts in time. Of course, we appreciate the ongoing support of Renate Dötzer and Frank Weinreich, WILEY-VCH, for this book series and their valuable collaboration in this project.

May 2006

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

A Personal Foreword

The dilemma of rapidly emerging fields is that reviews are often outdated before they are printed. To make a contribution that would endure, we knew we had to go beyond a snapshot of the current state of fragment-based drug discovery and instead provide a framework for upcoming advances. To achieve this goal, we needed to convince leading scientists to take time from their busy schedules to write chapters. Fortunately, nearly all those we approached agreed; and what you hold in your hands is a virtual, although not comprehensive, "Who's Who" in fragment-based drug discovery. We are extremely grateful to all of our contributors for the quality of their chapters.

One striking feature of this book is that more than half of the chapters come from industry-based researchers, and even many of the academic contributors have close ties to industry. It has been alleged that the best science is done in academia; this book proves that this is not necessarily the case. Indeed, industrial researchers have largely pioneered fragment-based drug discovery strategies. Part of the reason may be that many of the techniques involved require expensive equipment and infrastructure as well as large collaborations between scientists from disparate disciplines - collaborations that would be difficult to set up outside industry. The multidisciplinary nature of fragment-based approaches shows in this volume: contributors include computational chemists, NMR spectroscopists, X-ray crystallographers, mass-spectrometrists, as well as organic and medicinal chemists.

Although fragment-based strategies for drug discovery have now pervaded laboratories across the world, the ultimate success of any drug discovery technology is measured in the quantity and quality of drugs that it produces. Fragment-based drug discovery has only been practical for the past decade, too soon to expect it to produce marketed drugs, but we believe these will come in time. Moreover, many of the techniques and concepts described in this book will alter drug discovery endeavors in subtle, tangential ways. Ideally, readers will be inspired to improve the methods described here, or even to develop fundamentally new methods for fragment-based drug discovery. But even if this book only changes the way medicinal chemists approach lead optimization, or persuades them to look more closely at weak but validated hits, it will have served its purpose.

March 2006

Wolfgang Jahnke, Basel
Daniel A. Erlanson, San Francisco

List of Contributors

Cele Abad-Zapatero
Abbott Laboratories
Department of Structural Biology
R46Y, AP-10
100 Abbott Park Road
Abbott Park, IL 60064–6098
USA

Marcus D. Ballinger
Sunesis Pharmaceuticals, Inc.
341 Oyster Point Boulevard
South San Francisco, CA 94080
USA

Jeffrey M. Blaney
SGX Pharmaceuticals, Inc.
10505 Roselle Street
San Diego, CA 92121
USA

Stephen K. Burley
SGX Pharmaceuticals, Inc.
10505 Roselle Street
San Diego, CA 92121
USA

Thomas G. Davies
Astex Therapeutics Ltd
436 Cambridge Science Park
Milton Road
Cambridge, CB4 0QA
UK

Daniel A. Erlanson
Sunesis Pharmaceuticals, Inc.
341 Oyster Point Boulevard
South San Francisco, CA 94080
USA

Lara A. Estroff
Department of Materials Science and Engineering
Cornell University
214 Bard Hall
Ithaca, NY 14853
USA

Richard H. Griffey
SAIC, San Diego
10260 Campus Point Drive
San Diego, CA 92121
USA

Philip J. Hajduk
Abbott Laboratories
R46Y, AP 10
100 Abbott Park Road
Abbott Park, IL 60064–6098
USA

Matthias Hochgürtel
Alantos Pharmaceuticals AG
Im Neuenheimer Feld 584
69120 Heidelberg
Germany

Jeffrey R. Huth
Abbott Laboratories
R46Y AP 10
Abbott Park, IL 60064
USA

Wolfgang Jahnke
Novartis Institutes for Biomedical Research
Novartis Pharma AG
Lichtstrasse
4002 Basel
Switzerland

Harren Jhoti
Astex Therapeutics Ltd
436 Cambridge Science Park
Milton Road
Cambridge, CB4 0QA
UK

Martin Karplus
Department of Chemistry and Chemical Biology
Harvard University
12 Oxford Street
Cambridge, MA 02138
USA

Hartmuth C. Kolb
Department of Molecular and Medical Pharmacology
University of California
Los Angeles, CA 90095
USA

Vijay M. Krishnamurthy
Department of Chemistry and Chemical Biology
Harvard University
12 Oxford Street
Cambridge, MA 02138
USA

Jean-Marie Lehn
Laboratoire de Chimie Supramoléculaire
ISIS/ULP
8, allée Gaspard Monge
67083 Strasbourg Cedex
France

Carla Mattos
Department of Molecular and Structural Biochemistry
North Carolina State University
Campus Box 7622
128 Polk Hall
Raleigh, NC 27695
USA

Rob L. M. van Montfort
Astex Therapeutics Ltd
436 Cambridge Science Park
Milton Road
Cambridge, CB4 0QA
UK

Christopher W. Murray
Astex Therapeutics Ltd
436 Cambridge Science Park
Milton Road
Cambridge, CB4 0QA
UK

Vicki Nienaber
SGX Pharmaceuticals, Inc.
10505 Roselle Street
San Diego, CA 92121
USA

Tudor I. Oprea
Division of Biocomputing
University of New Mexico
School of Medicine
MSC11 6145
Albuquerque, NM 87131
USA

Dagmar Ringe
Departments of Biochemistry and Chemistry, and
Rosenstiel Basic Medical Sciences Research Center
Brandeis University, MS 029
415 South Street
Waltham, MA 02454-9110
USA

Stefanie Röper
Department of Chemistry
The Scripps Research Institute
10550 North Torrey Pines Road
La Jolla, CA 92037
USA

Daniel S. Sem
Chemical Proteomics Facility at Marquette
Department of Chemistry
Marquette University
535 North 14th Street
Milwaukee, WI 53233
USA

Miles Siegel
Discovery Chemistry Research, DC 1920
Lilly Research Laboratories
Indianapolis, IN 46285
USA

Geoffrey F. Stamper
Abbott Laboratories
Department of Structural Biology
R46Y, AP-10
100 Abbott Park Road
Abbott Park, IL 60064–6098
USA

Vincent S. Stoll
Abbott Laboratories
Department of Structural Biology
R46Y, AP-10
100 Abbott Park Road
Abbott Park, IL 60064–6098
USA

Collin M. Stultz
Division of Health Sciences and Technology, and
Department of Electrical Engineering and Computer Science
Massachusetts Institute of Technology, 32-310
77 Massachusetts Ave.
Cambridge, MA 02139
USA

Chaohong Sun
Abbott Laboratories
R46Y AP 10
Abbott Park, IL 60064
USA

Eric E. Swayze
Isis Pharmaceuticals
1896 Rutherford Rd.
Carlsbad, CA 92008
USA

Marcel L. Verdonk
Astex Therapeutics Ltd
436 Cambridge Science Park
Milton Road
Cambridge, CB4 0QA
UK

Michal Vieth
Discovery Chemistry Research, DC 1930
Lilly Research Laboratories
Indianapolis, IN 46285
USA

James A. Wells
Department of Pharmaceutical Chemistry & Molecular and Cellular Pharmacology
University of California, San Francisco
Box 2552, QB3
1700 4th Street
San Francisco, CA 94143-2552
USA

George M. Whitesides
Department of Chemistry and Chemical Biology
Harvard University
12 Oxford Street
Cambridge, MA 02138
USA

Glyn Williams
Astex Therapeutics Ltd
436 Cambridge Science Park
Milton Road
Cambridge, CB4 0QA
UK

Part I: Concept and Theory

1
The Concept of Fragment-based Drug Discovery

Daniel A. Erlanson and Wolfgang Jahnke

1.1
Introduction

Fragment-based drug discovery builds drugs from small molecular pieces. It combines the empiricism of random screening with the rationality of structure-based design. Though the concept was articulated decades ago, the approach has become practical only recently.

Historically, most drugs have been discovered by one of two methods. The first of these was famously summarized by Nobel Laureate Sir James Black, who noted that the best way to find a new drug is to start with an existing one. Indeed, any successful drug spawns a surge of similar molecules, as illustrated by the number of chemically similar COX-2 inhibitors or HIV protease inhibitors on the market and in development. Though often disparaged as "me-too" or "patent-busting", such efforts are productive. The first drug to market is rarely the best; one need only consider the state of HIV medication now compared to a decade ago to appreciate this fact. Even the search for new drugs often begins with known starting points in the form of natural ligands such as substrates, co-factors or inhibitors.

For diseases and targets where no drug or other starting point exists, the second major route of drug discovery, random screening, is essential. This approach to drug discovery is perhaps the oldest and most venerable but requires serendipity. Indeed, it was a serendipitous observation of bacterial killing by fungus that led Alexander Fleming to the discovery of the natural product penicillin. Many highly successful drugs, from cyclosporine to paclitaxel, have been discovered by screening collections of compounds. With each medicinal chemistry program, more chemical compounds and their analogs are added to corporate screening libraries.

The invention of combinatorial chemistry in the late 1980s and early 1990s vastly expanded the number of compounds in chemical collections, just as the development of sophisticated automation equipment and miniaturization of biological assays led to the advent of high-throughput screening, or HTS. Today, most major pharmaceutical companies and many biotechnology companies have in-house collections of hundreds of thousands or even millions of molecules.

In parallel to HTS, more rational routes for drug discovery have been sought. Structure-based drug design attempts to design inhibitors *in silico* on the basis of the three-dimensional structure of the target protein.

Among the latest developments in drug discovery is a concept called fragment-based drug design, or fragment-based screening (FBS). In contrast to conventional HTS, where fully built, "drug-sized" chemical compounds are screened for activity, FBS identifies very small chemical structures ("fragments") that may only exhibit weak binding affinity. Follow-up strategies are then applied to increase affinity by elaborating these minimal binding elements. Fragment-based drug design thus attempts to build a ligand piece-by-piece, in a modular fashion. Structural information plays a central role in most follow-up strategies. Therefore, fragment-based drug design can be viewed as the synthesis of random screening and structure-based design.

1.2
Starting Small: Key Features of Fragment-based Ligand Design

Fragment-based screening promises to have a great impact on drug discovery because of several advantages, which are summarized in the following sections.

1.2.1
FBS Samples Higher Chemical Diversity

Typical chemical libraries used for HTS contain 10^5 to 10^6 individual compounds. Though a million-compound library sounds vast, it covers only a very small portion of "drug space", the theoretical set of possible small, drug-like molecules. In fact, a widely quoted estimate (actually a back-of-the-envelope calculation in a footnote in a review of structure-based drug design) places this number at 10^{63} molecules [1], a number beyond the comprehension of anyone except perhaps astrophysicists. A recent estimate of the total number of molecules available for screening in all the commercial and academic institutions on the Earth is around 100 million, or 10^8, so even a planet-wide screening effort would not even scratch the surface of diversity space [2]. This will never change in any meaningful way. To understand why, imagine assembling a library of 10^{63} molecules. Even if miniaturization advances to the point where we need only 1 pmol of each molecule (about 0.5 ng for a 500-Da molecule), this would still require gathering 5×10^{47} tons of material, roughly 26 orders of magnitude larger than the mass of our planet. Clearly, libraries screened in HTS will always explore only a tiny fraction of drug space.

The explored fraction of diversity space swells when working with smaller molecules ("fragments"), because there are fewer possible small molecules than possible large molecules. If we screen small molecular fragments, rather than drug-sized molecules, we can cover exponentially larger swaths of diversity space with much smaller collections of molecules. To illustrate, imagine two sets of compounds, each

consisting of 1000 fragments. If we were to exhaustively make all binary combinations with a single asymmetric linker, this would yield (1000 molecules) × (1000 molecules) = 1 000 000 molecules to synthesize and screen, a daunting task. In contrast, if we could identify the five best fragments in each set and only combine and screen those, we would only need to synthesize and test [(1000 molecules) + (1000 molecules)] + [(5 molecules) × (5 molecules)] = 2025 molecules. This number is clearly much more manageable, and still covers the same chemical diversity space.

A first-principles computational analysis suggests that there are roughly 13.9×10^6 stable, synthetically feasible small molecules with a molecular weight less than or equal to 160 Da (44×10^6 once stereoisomers are considered, although the approach excludes compounds containing three- and four-membered rings and elements other than carbon, hydrogen, oxygen, nitrogen, and halogens) [3]. This is still a large number, but it is at least a comprehensible number, especially compared with 10^{63}. It shows that, with fragment-based screening, a higher (although still very small) proportion of diverse drug space can be covered. From a technical standpoint as well, focusing on these smaller fragments could simplify many aspects of the drug discovery process, from compound acquisition and synthesis through data management.

1.2.2 FBS Leads to Higher Hit Rates

Imagine a small fragment with high but imperfect complementarity to a target protein. Now imagine adding a methyl group at exactly the right spot to increase complementarity even further: rendering the fragment more complex in the right manner leads to slightly increased affinity to the target protein. But imagine adding the methyl group at any other spot, so that it protrudes from this fragment towards the receptor such that the modified fragment can no longer bind to the target: rendering the fragment more complex in the "wrong" manner ablates affinity for the receptor. Notably, there are many more ways to increase complexity in the "wrong" manner, and doing so often leads to a decrease of binding affinity by several orders of magnitude, whereas in the lucky case of increasing complexity in the "right" manner, binding is generally only enhanced by one or two orders of magnitude. This simple example makes sense intuitively, and a more rigorous theoretical analysis comes to the same conclusion: as molecules become more complex, additional chemical groups are much more likely to ablate binding than to enhance it [4]. The probability of binding (the "hit rate" in screening) thus decreases with increasing ligand complexity. Libraries containing smaller compounds ("fragments") are expected to exhibit higher hit rates, although the resulting affinities are generally weak and so require sensitive detection methods.

1.2.3
FBS Leads to Higher Ligand Efficiency

Screening drug-sized molecules is thought to favor ligands with several sub-optimal binding interactions, rather than those with a few optimal interactions. This is schematically shown in Fig. 1.1: the drug-sized molecule on the left side is identified by HTS since it binds to the receptor. However, none of the binding interactions are optimal, since establishing one optimal interaction would disrupt another interaction. All binding interactions are thus compromised and do not retain the full strength they would have without the molecular strain.

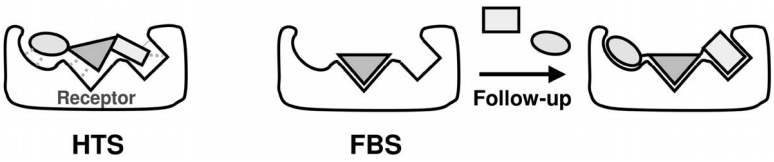

Fig. 1.1
Potential drawback of HTS (left), and principle and advantages of FBS (right): In HTS, fully assembled, "drug-sized" ligands are identified, but with multiple compromised, non-optimal binding interactions. In FBS, ligands for individual subpockets are identified separately, and show few but good binding interactions. Follow-up strategies such as fragment elaboration or linking are used to increase ligand affinity.

Relative to their molecular size, fragments can thus show more favorable binding energies than drug-sized molecules. The binding energy, normalized by the number of heavy atoms in the ligand, is referred to by the term ligand efficiency [5]. Smaller fragments can have higher ligand efficiency, leading to smaller drugs with better chances for favorable pharmacokinetics [6, 7]. This concept is also being applied to conventional HTS with the advent of "lead-like", instead of "drug-like," compound libraries [8].

1.3
Historical Development

The basic concept of fragment-based drug discovery was developed about 25 years ago by William Jencks, who wrote in 1981 that the affinities of whole molecules could be understood as a function of the affinities of separate parts:

"It can be useful to describe the Gibbs free energy changes for the binding to a protein of a molecule, A–B, and of its component parts, A and B, in terms of the "intrinsic binding energies" of A and B (ΔG^{i}_{A} and ΔG^{i}_{B}) and a "connection Gibbs energy" (ΔG^{s}) that is derived largely from changes in translational and rotational entropy [9]."