Natural Product Chemistry for Drug Discovery

Edited by Antony D Buss and Mark S Butler

RSC Biomolecular Sciences

RSC Publishing
Natural Product Chemistry for Drug Discovery
RSC Biomolecular Sciences

Editorial Board:
Professor Stephen Neidle (Chairman), The School of Pharmacy, University of London, UK
Dr Marius Clore, National Institutes of Health, USA
Professor Roderick E Hubbard, University of York and Vernalis, Cambridge, UK
Professor David M J Lilley FRS, University of Dundee, UK

Titles in the Series:
1: Biophysical and Structural Aspects of Bioenergetics
2: Exploiting Chemical Diversity for Drug Discovery
3: Structure-based Drug Discovery: An Overview
4: Structural Biology of Membrane Proteins
5: Protein–Carbohydrate Interactions in Infectious Disease
6: Sequence–specific DNA Binding Agents
7: Quadruplex Nucleic Acids
8: Computational and Structural Approaches to Drug Discovery: Ligand–Protein Interactions
9: Metabolomics, Metabonomics and Metabolite Profiling
10: Ribozymes and RNA Catalysis
11: Protein–Nucleic Acid Interactions: Structural Biology
12: Therapeutic Oligonucleotides
13: Protein Folding, Misfolding and Aggregation: Classical Themes and Novel Approaches
14: Nucleic Acid–Metal Ion Interactions
15: Oxidative Folding of Peptides and Proteins
16: RNA Polymerases as Molecular Motors
17: Quantum Tunnelling in Enzyme-Catalysed Reactions
18: Natural Product Chemistry for Drug Discovery

How to obtain future titles on publication:
A standing order plan is available for this series. A standing order will bring delivery of each new volume immediately on publication.

For further information please contact:
Sales and Customer Care, Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge, CB4 0WF, UK
Telephone: + 44 (0)1223 432360, Fax: + 44 (0)1223 420247, Email: sales@rsc.org
Visit our website at http://www.rsc.org/Shop/Books/
Natural Product Chemistry for Drug Discovery

Edited by

Antony D. Buss and Mark S. Butler
MerLion Pharmaceuticals, Singapore
Preface

Natural products hold a special place in drug discovery having provided and inspired numerous life saving medicines and medical breakthroughs, particularly in the treatment of infectious diseases, cancer, hypercholesterolemia and immunological disorders. Twenty one drugs approved for marketing between 2003 and 2008 owe their existence to natural product leads discovered from mainly actinomycete, bacteria and fungal sources.\(^1\) It has been our intention with this book to not only provide insights into the likely sources and methodologies that may be used to discover new natural product based drugs in the future, but also to stress the utility and importance of this approach to drug discovery in terms of new clinical candidates and recent commercial successes.

The final section of this book provides fascinating accounts of the twists, turns and pitfalls, as well as the role serendipity played, in the successful development and commercialisation of daptomycin and micafungin. Accounts of natural product derived drug candidates which are currently being evaluated in clinical trials may be found in Chapters 11–13, with salinosporamide A and bevirimat described in detail. The pipeline of 36 drug candidates which are in late stage clinical development may imply a continuing role for natural products in drug discovery, but we will return to this issue towards the end of this preface. Before then, let us look at the earlier chapters which follow in this book.

Well known for their thorough analyses of the sources of new and approved drugs, Newman and Cragg set the scene in Chapter 1 with a discussion on the historical influence natural products have had on the drug discovery process, with particular emphasis on antibacterial, antifungal and anticancer agents. The reason for the success of natural product chemistry in drug discovery is multifactorial, but certainly includes the unique “chemical space” that is occupied by such molecules. A particularly elegant account of how this applies to the required physicochemical property space for antibacterial compounds
has been made recently by O'Shea and Moser. In Chapter 2, Singh and Culberson expand on this theme with a comparison of the diversity of natural products with various synthetic compound libraries and their impact as drug leads in general.

La Clair, in Chapter 3, adopts a cinematic approach whilst delving into the mechanistic modes of action and the complex roles that natural products play. Included in this account are descriptions of how natural products have led to a better understanding of the regulation of tubulin and actin assembly in tumour cells and to the identification of an array of new, putative anticancer drug targets.

In Chapter 4, Cordell thoroughly evaluates the impact of the Convention on Biological Diversity (CBD) and other related agreements on academic and industrial natural product research. While the CBD has resulted in the development of laws and practices that have protected the sovereign rights of countries over their genetic resources, it has also led to natural product research programmes being compromised in scope and has perhaps contributed, at least in part, to many pharmaceutical companies terminating their natural product research activities.

Plants, microorganisms and, to a lesser extent, macromarines have been the main sources of natural product based drugs (produced as secondary metabolites). Reviews of these traditional sources of naturally occurring chemical compounds are found in Chapters 5–7, together with hints and suggestions as to how these sources may be better utilised to continue supplying new drug leads in the future.

Advances in high throughputscreening technology, particularly with regard to detection methods and readouts, are reviewed in Chapter 8. These advances in biological screening, coupled with improvements in chromatographic and analytical techniques (highlighted in Chapter 9), have led to a significant reduction in the time required to purify active compounds from complex mixtures and to determine their chemical structures. In addition to conventional natural product discovery approaches, new versions of two major classes of natural products, the non-ribosomal peptides and polyketides, can now be engineered and produced using genetic manipulation techniques because of the ability to correlate gene sequence with amino acid sequence and thus, the chemical structure of the biosynthetic product. In Chapter 10, Udwary reviews the advances made in this field of combinatorial biosynthesis over the last 15 years, together with an account of some of the significant technical limitations that still need to be overcome before the rational engineering of biosynthetic pathways can be more readily harnessed for drug discovery.

We promised to return to the earlier statement that a healthy development pipeline of natural product derived candidates implies that natural products will still have a role to play in modern day drug discovery. In fact, this is far from reality. Firstly, these late-stage clinical candidates reflect the output from research activities undertaken at least 10 years ago and certainly not the current situation. Secondly, there is a lack of truly novel chemical templates in the pipeline and thirdly, it is clear that very few pharmaceutical companies remain engaged, at least internally, in natural product drug discovery activities.
In 2007, the US Food and Drug Administration approved only 16 new molecular entities, the lowest in a single year since 1983.\textsuperscript{3} Despite a slight improvement in 2008, there remains a disturbing overall decline in pharmaceutical R&D productivity that is exacerbated by exponential rises in R&D costs, erosion of sales as many key products face patent expiration and increasing regulatory hurdles. With a burgeoning and aging population, the need for innovative new medicines throughout the world will not diminish. So is there a place for natural product based drug discovery in the future and, if so, where will new biologically active natural products come from?

In this book many of the significant technical advances which have accelerated the screening, purification and structural identification of bioactive natural products have been highlighted. As Bugni \textit{et al}. remind us in Chapter 9, many of the previous bottlenecks that made natural products discovery a slow, laborious process have indeed been removed. However, for natural product based drug discovery to become cost effective and remain competitive, a number of key problems must be addressed, including the continual discovery of known compounds from existing natural product extract collections, the scarcity of novel bioactive chemical templates and the challenge of structurally modifying sometimes complex, often oxygen-rich, chiral natural product lead structures.

With the concept that secondary metabolites have evolved to specifically interact with protein targets and that these are not so different from human proteins, the construction of synthetic compound libraries inspired or based on natural product templates will continue to gain popularity and general acceptance as a valid drug discovery approach. Given that access to biologically relevant, drug-like chemical space is central to the drug discovery process and that natural products often occupy very different areas of this “space” compared to synthetic compounds,\textsuperscript{4} then we believe that the search for drug leads from natural products offers a complimentary and much needed approach to other drug discovery strategies.

Antony D. Buss and Mark S. Butler
MerLion Pharmaceuticals, Singapore

References
## Contents

### Section 1 Introduction to Natural Products for Drug Discovery

#### Chapter 1 Natural Products as Drugs and Leads to Drugs: The Historical Perspective

*David J. Newman and Gordon M. Cragg*

1. Ancient History (>2900 BCE to 1800 CE) ........................................... 3  
2. The Initial Influence of Chemistry upon Drug Discovery ............... 6  
   2.1 Alkaloids .................................................................................. 6  
   2.2 Aspirin ..................................................................................... 8  
   2.3 Digitalis .................................................................................. 9  
3. 20th and 21st Century Drugs/Leads from Nature ...................... 10  
   3.1 Antibacterial and Antifungal Antibiotics .................................. 10  
   3.2 Antiviral Agents .................................................................... 19  
   3.3 Natural Product Based Antitumour Agents ......................... 21  
4. Final Comments ............................................................................. 23  
References ....................................................................................... 24

#### Chapter 2 Chemical Space and the Difference Between Natural Products and Synthetics

*Sheo B. Singh and J. Chris Culberson*

1. Introduction .................................................................................. 28  
2. Sources of Organic Compounds and Drug Leads ...................... 29  
   2.1 Natural Products ................................................................... 29  
   2.2 Natural Product Derivatives .................................................. 29  
3. Synthetic Compounds ................................................................ 30  
   3.1 Synthetic Compound Libraries ............................................. 30  
   3.2 Combinatorial Libraries ......................................................... 30

---

RSC Biomolecular Sciences No. 18  
Natural Product Chemistry for Drug Discovery  
Edited by Antony D. Buss and Mark S. Butler  
© Royal Society of Chemistry 2010  
Published by the Royal Society of Chemistry, www.rsc.org  
ix
Chapter 3  Mechanism of Action Studies

James J. La Clair

1  Introduction 44
2  Some Like It Hot: Esperamicin A1, Neocarzinostatin and Related Enediyne Antibiotics 45
3  To Catch a Mockingbird: Taxol, Epothilone and the Microtubule 47
4  Notorious: Jasplakinolide, Alias Jaspamide and Actin 51
5  Invasion of the Pathway Snatchers: Artemisinin 53
6  Once Upon a Time in the Immune System: FK-506, Cyclosporin A and Rapamycin 55
7  Back to the Cytoskeleton: the Phorboxazoles 56
8  It’s a Wonderful Target: VTPase and its Targeting by Apicularen A, Salicylihalamide A and Palmerolide A 59
9  Double Indemnity: Bistramide A 61
10  The Matrix: the Pladienolides and Splicing Factor SF3b 62
11  The Unusual Suspects: (+)-Avrainvillamide 65
12  Close Encounters of a Third Kind: Ammosamides, Blebbestatin and Myosin 67
13  The End 69
References 69
Section 2 Sources of Compounds

Chapter 4 The Convention on Biological Diversity and its Impact on Natural Product Research
Geoffrey A. Cordell

1 Introduction 81
2 Historical Perspective 85
3 The Convention on Biological Diversity 87
4 Implementation and Regulatory Outcomes of the CBD 92
5 Assessment of Impact 95
  5.1 An Overview and Some Examples 95
  5.2 An Informal Survey 100
  5.3 Survey Results 101
  5.4 Survey Overview 116
6 The TRIPS Agreement and the CBD 116
7 Other Aspects and Outcomes 123
  7.1 The International Cooperative Biodiversity Group Programme 125
8 Some Recommendations 127
9 A Web of Interconnectedness 130
10 A Different World 131
11 Conclusions 133
Acknowledgements 134
References 135

Chapter 5 Plants: Revamping the Oldest Source of Medicines with Modern Science
Giovanni Appendino and Federica Pollastro

1 Introduction 140
2 Plant Secondary Metabolites vs. Secondary Metabolites of Other Origin 143
3 Unnatural Sources of Plant Secondary Metabolites 146
4 Critical Issues in Plant-based Natural Product Drug Discovery 149
  4.1 Intellectual Property (IP) Issues 149
  4.2 Pleiotropy and Synergy 151
  4.3 Extract Libraries vs. Fraction (Peak) Libraries vs. Compound Libraries 153
  4.4 Removal of Interfering Compounds 155
5 Selection Strategies for Plant-Based Natural Product Drug Discovery 156
  5.1 Ethnopharmacology 156
# Contents

5.2 Zoopharmacy and Animal Toxicology 157  
5.3 Traditional Medicine 158  
5.4 Dietary Plants and Spices 159  
6 The Pharmaceutical Relevance of Plants 161  
6.1 Plants as a Source of Lead Structures and Drugs 161  
6.2 Plants as a Source of Standardised Extracts 163  
7 Conclusions 167  
References 168  

Chapter 6  Macromarines: A Selective Account of the Potential of Marine Sponges, Molluscs, Soft Corals and Tunicates as a Source of Therapeutically Important Molecular Structures  
*Jennifer Carroll and Phillip Crews*

1 Introduction 174  
1.1 Macroorganisms: Outstanding Success in Producing Viable Drug Leads 175  
1.2 Setting that Ara A and Ara C Story Straight 175  
1.3 The Potential Role of Invertebrate Associated Microorganisms and Secondary Metabolite Production 176  
1.4 Macromarine Evolution 176  
2 Sponges 177  
2.1 Natural History of Sponges—a Primitive Phylum with Remarkable Biosynthetic Capabilities 177  
3 Molluscs 186  
3.1 Natural History of Molluscs—the Source of Numerous Preclinical Drug Leads 186  
4 Soft Corals 189  
4.1 Natural History of Cnidarians—the “Stinging Nettle” of the Sea 189  
5 Tunicates 192  
5.1 Natural History of Tunicates—Our Closest Marine Invertebrate Relations 192  
6 Conclusions 194  
References 195  

Chapter 7  Microorganisms: Their Role in the Discovery and Development of Medicines  
*Cedric Pearce, Peter Eckard, Iris Gruen-Wollny and Friedrich G. Hansske*

1 Introduction 215  
2 Bacteria 218  
3 Fungi 220  
4 Terrestrial and Marine Microorganisms 221
Section 3 Advances in Technology

Chapter 8 Advances in Biological Screening for Lead Discovery
Christian N. Parker, Johannes Ottl, Daniela Gabriel and Ji-Hu Zhang

1 Introduction 245
   1.1 Natural Product Screening and the Development of HTS 247
   1.2 Chapter Objectives 247
2 Types of HTS Assays 247
   2.1 In vitro Biochemical Assays 248
   2.2 Cell-based Assays 255
   2.3 Modelling to Identify False Positives and Negatives 261
3 Emerging Trends 262
   3.1 New HTS Approaches 262
Acknowledgements 265
References 265

Chapter 9 Advances in Instrumentation, Automation, Dereplication and Prefractionation
Tim S. Bugni, Mary Kay Harper, Malcolm W.B. McCulloch and Emily L. Whitson

1 Introduction 272
2 Dereplication 274
3 Extraction 275
4 Prefractionation 276
5 Isolation and Purification 278
5.1 Automated Purification 279
6 HPLC Separation Technologies 279
7 Mass Spectrometry 282
8 NMR 285
8.1 Probe Technology 285
8.2 Structure Elucidation 287
8.3 Methods for Fast NMR 288
8.4 Automated Structure Elucidation 290
8.5 Configuration by NMR 291
8.6 Residual Dipolar Couplings 292
9 Conclusions 292
References 293

Chapter 10 Natural Product Combinatorial Biosynthesis: Promises and Realities

Daniel W. Udwary

1 Introduction 299
2 A Brief History of Natural Product Biosynthesis 300
3 Promises 304
4 Realities 307
5 Future Biotechnological Promises 312
References 314

Section 4 Natural Products in Clinical Development

Chapter 11 A Snapshot of Natural Product-Derived Compounds in Late Stage Clinical Development at the End of 2008

Mark S. Butler

1 Introduction 321
2 NP-derived Drugs Launched in the Last Five Years 324
3 Late Stage NDAs and Clinical Candidates 327
3.1 Antibacterial 327
3.2 Oncology 332
3.3 Other Therapeutic Areas 340
4 Conclusions and Outlook 342
References 343