

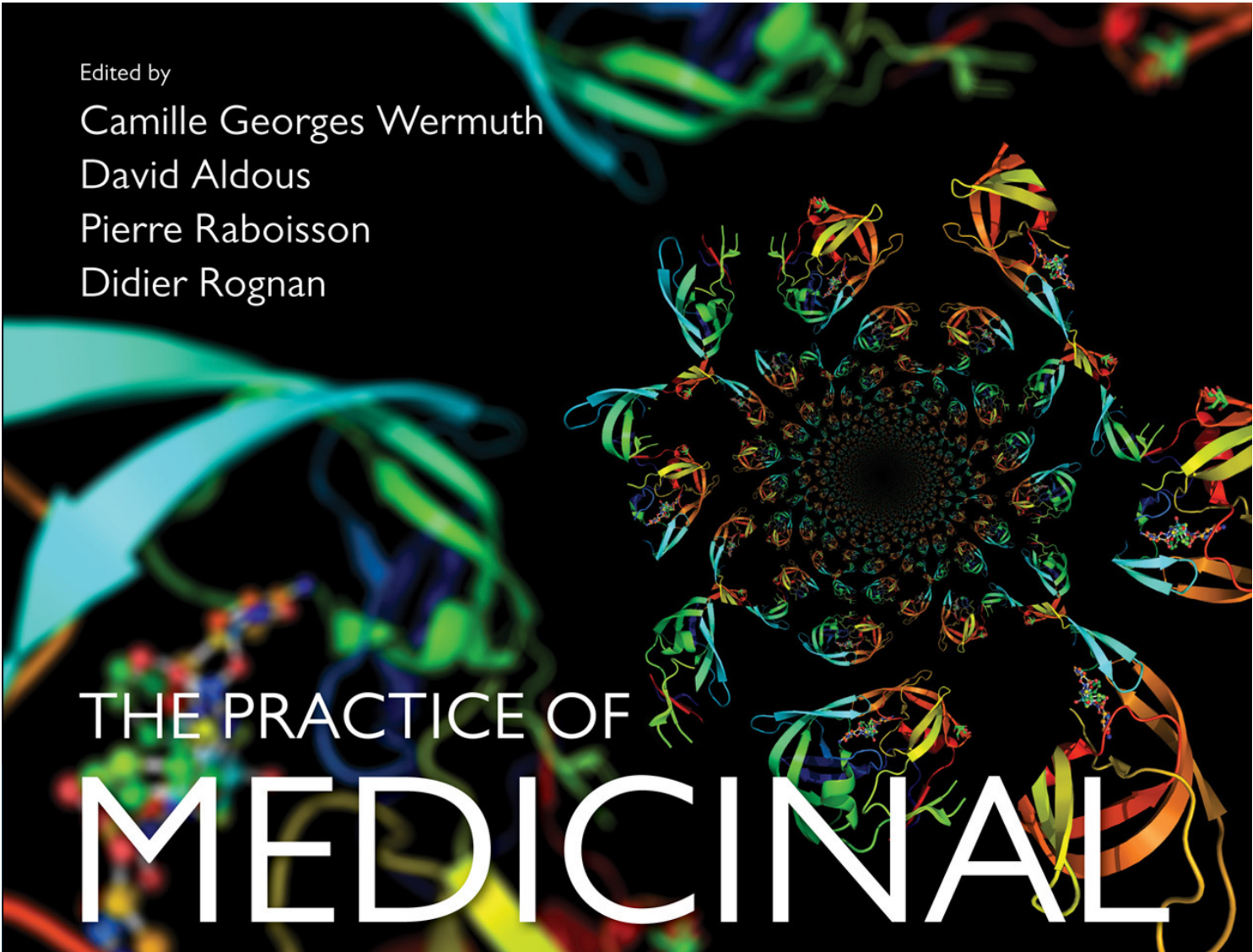
Edited by

Camille Georges Wermuth

David Aldous

Pierre Raboisson

Didier Rognan



THE PRACTICE OF MEDICINAL CHEMISTRY



FOURTH EDITION



THE PRACTICE OF MEDICINAL CHEMISTRY

FOURTH EDITION

Copyright Elsevier 2026

This page intentionally left blank

The Practice of Medicinal Chemistry

FOURTH EDITION

Edited by

CAMILLE GEORGES WERMUTH, PhD, PHARM D

Founder of Prestwick Chemical, Strasbourg, France

DAVID ALDOUS, BSC, DPHIL

Boston Head Lead Generation to Candidate Realisation, Boston, MA, USA

PIERRE RABOISSON, PhD, PHARM D

Janssen Infectious Diseases and Diagnostics BVBA, Beerse, Belgium

DIDIER ROGNAN, PhD, PHARM D

Laboratoire d'Innovation Thérapeutique, CNRS-Université de Strasbourg, Illkirch, France



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

Academic Press is an imprint of Elsevier



Academic Press is an imprint of Elsevier
125, London Wall, EC2Y 5AS
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 Ltd. All rights reserved.

First edition 1996
Reprinted 2001
Second edition 2003
Third edition 2008

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 may 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.

Library of Congress Cataloging-in-Publication Data

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

British Library Cataloguing-in-Publication Data

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

ISBN: 978-0-12-417205-0

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

		Working together to grow libraries in developing countries
www.elsevier.com • www.bookaid.org		

Contents

List of Contributors	xi	VII. Target Validation	68
Foreword	xiii	VIII. Conclusion	68
Preface to the Fourth Edition	xv	References	68
Preface to the Third Edition	xvii		
Preface to the Second Edition	xix		
Preface to the First Edition	xxi		

I

GENERAL ASPECTS OF MEDICINAL CHEMISTRY

1. Medicinal Chemistry: Definitions and Objectives, Drug Activity Phases, Drug Classification Systems

PETER IMMING

I. Definitions and Objectives	3
II. Drug Activity Phases	7
III. Drug Classification Systems	8
References	12

2. Evaluation of the Biological Activity of Compounds: Techniques and Mechanism of Action Studies

IAIN G. DOUGALL AND JOHN UNITT

I. Introduction	15
II. Drug Discovery Approaches and Screening Cascades	16
III. <i>In Vitro</i> Assays	17
IV. <i>Ex Vivo</i> Assays	38
V. <i>In Vivo</i> Assays	39
Acknowledgements	42
References	42

3. Drug Targets, Target Identification, Validation, and Screening

WALTER M.M. VAN DEN BROECK

I. Introduction	45
II. What is a Drug Target?	46
III. The Purpose of Target Identification	47
IV. Target Options and Treatment Options	51
V. Target Deconvolution and Target Discovery	53
VI. Methods for Target Identification and Validation	54

II

LEAD COMPOUND DISCOVERY STRATEGIES

4. Strategies in the Search for New Lead Compounds or Original Working Hypotheses

CAMILLE G. WERMUTH, BRUNO VILLOUTREIX, SERGE GRISONI,
ANNE OLIVIER AND JEAN-PHILIPPE ROCHER

I. Introduction	73
II. First Strategy: Analog Design	74
III. Second Strategy: Systematic Screening	78
IV. Third Strategy: Exploitation of Biological Information	84
V. Fourth Strategy: Planned Research and Rational Approaches	90
VI. Fifth Strategy: Applying Biophysical Technologies and Computational Methods	93
VII. Conclusion	96
References	96

5. Natural Products as Pharmaceuticals and Sources for Lead Structures

DAVID J. NEWMAN, GORDON M. CRAGG AND DAVID G.I. KINGSTON

I. Introduction	102
II. The Importance of Natural Products in Drug Discovery and Development	102
III. The Design of an Effective Natural-Products-Based Approach to Drug Discovery	106
IV. Examples of Natural Products or Analogues as Drugs	116
V. Future Directions in Natural Products as Drugs and Drug Design Templates	128
VI. Summary	130
References	131

6. *In Silico* Screening: Hit Finding from Database Mining

TIAGO RODRIGUES AND GISEBERT SCHNEIDER

I. Introduction	141
II. <i>In Silico</i> Screening	143
III. <i>De Novo</i> Design	150

IV. Conclusions and Future Directions	154
Glossary	156
References	156

7. Fragment-Based Drug Discovery

VENKATA VELVADAPU, BENNETT T. FARMER AND ALLEN B. REITZ

I. Ligand–Protein Interactions: First Principles	162
II. What is Fragment-Based Drug Discovery?	164
III. Creation and Analysis of FBDD Libraries	166
IV. Fragment Screening Methods	167
V. Other Biochemical and Biophysical Methods	171
VI. Fragment Merging/Linking/Growing	172
VII. Fragment Hit Follow-Up, and Pitfalls to Avoid	174
VIII. Zelboraf [®] , First Approved Drug from FBDD	175
IX. Limitations of FBDD	176
X. Trends for the Future	176
References	177

8. Molecular Variations Based on Isosteric Replacements

PAOLA CIAPETTI AND BRUNO GIETHLEN

I. Introduction	181
II. History: Development of the Isosterism Concept	182
III. Currently Encountered Isosteric and Bioisosteric Modifications	186
IV. Scaffold Hopping	220
V. Analysis of the Modifications Resulting from Isosterism	224
VI. Minor Metalloids-Toxic Isosteres	229
References	233

9. Ring Transformations

CHRISTOPHE MORICE AND CAMILLE G. WERMUTH

I. Introduction	243
II. Analogical Approaches	244
III. Disjunctive Approaches	258
IV. Conjunctive Approaches	260
V. Conclusion	263
References	263

10. Macrocycles: Under-Explored and Poorly Exploited Drug Class Despite the Proven Therapeutic Potential

PIERRE RABOISSON

I. Nature as a Source of Macrocycles	267
II. Identification of Macrocyclic Drugs Using Either Phenotypic Screen or Target-Based Approach	268
III. Macrocycles: The Drugs in the Middle Space	270
IV. Effect of the Macrocyclization on Drug-Like Properties	270
V. Interaction of Macrocycles with their Targets	271
VI. Synthesis of Macrocycles & Library Enrichment	274
VII. Conclusion	274
References	274

III

PRIMARY EXPLORATION OF STRUCTURE-ACTIVITY RELATIONSHIPS

11. Conformational Restriction and Steric Hindrance in Medicinal Chemistry

PETER WIPF, ERIN M. SKODA AND ANDRÉ MANN

I. Introduction	279
II. Case Studies	285
III. Summary and Outlook	297
References	297

12. Application Strategies for the Primary Structure–Activity Relationship Exploration

CAMILLE G. WERMUTH, SERGE GRISONI, BRUNO VILLOUTREIX AND JEAN-PHILIPPE ROCHER

I. Introduction	302
II. Preliminary Considerations	302
III. Hit Optimization Strategies	303
IV. Application Rules	312
References	317

13. Substituent Groups

PATRICK BAZZINI AND CAMILLE G. WERMUTH

I. Introduction	319
II. Methyl Groups	320
III. Effects of Unsaturated Groups	331
IV. Effects of Halogenation	338
V. Effects of Hydroxylation	345
VI. Effects of Thiols and Other Sulfur-Containing Groups	346
VII. Acidic Functions	349
VIII. Basic Groups	351
IX. Attachment of Additional Binding Sites	352
References	354

14. The Role of Functional Groups in Drug–Receptor Interactions

LAURENT SCHAEFFER

I. Introduction	359
II. General Principles	360
III. The Importance of the Electrostatic and Steric Match Between Drug and Receptor	360
IV. The Strengths of Functional Group Contributions to Drug–Receptor Interactions	370
V. Cooperative Binding	376
References	377

15. Compound Properties and their Influence on Drug Quality

HONGMING CHEN, OLA ENKVIST AND THIERRY KOGEJ

I. Introduction	379
II. Compound Drug-Likeness Analysis	380
III. Compound Promiscuity	382
IV. Compound ADMET Properties	384
V. Ligand Binding Efficiency Metrics	386
VI. Conclusions and Future Outlook	390
References	391

16. Pharmacological Space

ANDREW L. HOPKINS

I. What is Pharmacological Space?	395
II. Chemical Space	395
III. Target Space	399
IV. Conclusions	405
Acknowledgments	407
References	407

17. Systems Biology: A New Paradigm for Drug Discovery

HIBA ABI HUSSEIN, ALEXANDRE BORREL, LESLIE REGAD, DELPHINE FLATTERS, ANNE BADEL, COLETTE GENEIX, MICHEL PETITJEAN, ANNE-CLAUDE CAMPROUX AND OLIVIER TABOUREAU

I. Introduction	409
II. Drug-Target Space (off-target)	413
III. Systems Biology Space	415
IV. Phenotype Space	418
V. Examples	418
VI. Conclusion	421
References	421

IV

SUBSTITUENTS AND FUNCTIONS: QUALITATIVE ASPECTS OF STRUCTURE-ACTIVITY RELATIONSHIPS

18. Optical Isomerism in Drugs

CAMILLE G. WERMUTH

I. Introduction	429
II. Experimental Facts and their Interpretation	430
III. Optical Isomerism and Pharmacodynamic Aspects	435
IV. Optical Isomerism and Pharmacokinetic Aspects	437
V. Practical Considerations	439
References	444

19. Multitarget Drugs: Strategies and Challenges for Medicinal Chemists

RICHARD MORPHY AND ZORAN RANKOVIC

I. Introduction	449
II. Strategies for Lead Generation	451
III. Main Areas of Focus in Discovery	453
IV. Optimization of the Activity Profile and Wider Selectivity	465
V. The Physicochemical Challenge	467
VI. Summary	469
References	470

20. Selective Optimization of Side Activities (SOSA) in Drug Discovery

TIM JONCKERS

I. Introduction	473
II. Ritonavir: Rejuvenating a Suboptimal Drug	474
III. Sildenafil, Side Effects are Not Always Bad	477
IV. Nucleotide Prodrugs: Chemical Trojan Horses	478
V. Miltefosine	481
VI. Aztreonam	483
VII. Conclusions	485
References	485

V

SPATIAL ORGANIZATION, RECEPTOR MAPPING AND MOLECULAR MODELING

21. Pharmacophore Identification and Pseudo-Receptor Modeling

GERHARD WOLBER AND WOLFGANG SIPPL

I. Introduction	489
II. Methodology	493
III. Advanced Approaches	497
IV. Application Study: Novel Histamine H ₃ -Receptor Antagonists	501
V. Recent Developments and Outlook	505
VI. Conclusions	507
References	507

22. Protein Crystallography and Drug Discovery

JEAN-MICHEL RONDEAU AND HERMAN SCHREUDER

I. Introduction	511
II. Historical Background	512
III. Basic Principles and Methods of Protein Crystallography	514
IV. Applications	527
V. Two Selected Examples	531
VI. Outlook	532
References	533

23. Physiological Aspects Determining the Pharmacokinetic Properties of Drugs

KOEN BOUSSERY, FRANS M. BELPAIRE AND JOHAN VAN DE VOORDE

I. Introduction	539
II. Passage of Drugs Through Biological Barriers	541
III. Drug Absorption	542
IV. Drug Distribution	547
V. Drug Elimination	548
VI. Some Pharmacokinetic Parameters and Terminology	552
VII. Variability in Pharmacokinetics	557
Further Reading	559

24. Biotransformation Reactions and their Enzymes

BERNARD TESTA AND BERND CLEMENT

I. Introduction	561
II. Functionalization Reactions	563
III. Conjugation Reactions	571
IV. Biological Factors Influencing Drug Metabolism	579
V. What is the Relative Significance of These Many Types of Metabolic Reactions?	580
VI. Concluding Remarks	581
References	582

25. Biotransformations Leading to Toxic Metabolites: Chemical Aspects

ANNE-CHRISTINE MACHERY AND PATRICK M. DANSETTE

I. Historical Background	585
II. Introduction	586
III. Reactions Involved in Bioactivation Processes	587
IV. Examples of Metabolic Conversions Leading to Toxic Metabolites	599
V. Conclusion	608
Acknowledgments	611
References	611

26. Drug Transport Mechanisms and their Impact on the Disposition and Effects of Drugs

JEAN-MICHEL SCHERRMANN

I. Introduction	615
II. Biology and Function of Transporters	616
III. Transporters in Drug Disposition	620
IV. Roles of Transporters in Drug Pharmacokinetics, Pharmacodynamics and Toxicology	624
V. Conclusion	628
Acknowledgments	628
References	628

27. Strategies for Enhancing Oral Bioavailability and Brain Penetration

GERHARD GROSS

I. Introduction	631
-----------------	-----

II. Enhancing Oral Bioavailability	632
III. Enhancing Brain Penetration	645
References	653

28. Designing Prodrugs and Bioprecursors

YONG MI CHOI-SLEDESKI AND CAMILLE G. WERMUTH

I. Introduction	657
II. The Different Kinds of Prodrugs	658
III. Practical Applications of Carrier Prodrugs	661
IV. Unique Approaches to Carrier Prodrug Design	668
V. Bioprecursor Prodrug Examples	686
VI. Discussion	690
VII. Difficulties and Limitations	690
VIII. Conclusion	691
References	692

VI

CHEMICAL MODIFICATIONS INFLUENCING THE PHARMACOKINETIC PROPERTIES

29. Drug Delivery with Organic Solvents or Colloidal Dispersed Systems

BERND U. RIEBESEHL

I. Introduction	699
II. Physicochemical Drug Properties	700
III. Oral Drug Delivery	700
IV. Parenteral Drug Delivery	704
References	720

30. Preparation of Water-Soluble Compounds by Covalent Attachment of Solubilizing Moieties

CAMILLE G. WERMUTH AND DOMINIQUE LESUISSE

I. Introduction	723
II. Solubilization Strategies	724
III. Acidic Solubilizing Chains	726
IV. Basic Solubilizing Chains	734
V. Nonionizable Side Chains	739
VI. Concluding Remarks	741
References	742

31. Improving the Water-Solubility of Compounds by Molecular Modification to Disrupt Crystal Packing

MINORU ISHIKAWA AND YUICHI HASHIMOTO

I. Introduction	747
II. Rationale for Disruption of Crystal Packing as an Alternative Method to Improve Solubility	748
III. Improvement of Solubility by Disrupting Intermolecular Hydrogen Bonds	749

	CONTENTS	ix
IV. Improvement of Solubility by Disrupting Molecular Planarity	751	808
V. Improvement of Solubility by Bending the Molecular Structure	760	819
VI. Advantages of Improving Solubility by Molecular Modification to Weaken Intermolecular Interaction	762	819
VII. Conclusion	763	820
References	764	820
 32. Chemical and Physicochemical Approaches to Solve Formulation Problems		
HARVEY LIEBERMAN AND N. MURTI VEMURI		
I. Introduction	767	
II. Stability	767	
III. Bioavailability	774	
IV. Modifying the Duration of Action	781	
V. Manufacturing Issues	782	
VI. Adapting to Patient's Needs	783	
References	788	
 33. Discover a Drug Substance, Formulate, and Develop It to a Product		
PIERRE deMONTIGNY, DAVID HARRIS, CHRIS HO, FRANZ WEIBERTH, BRUNO GALLI AND BERNARD FALLER		
I. Introduction	793	
II. The Discovery Phase	794	
III. Defining Experimental Formulations, the Creative Phase	796	
IV. Preparation for a New Drug-Product Launch	800	
V. Conclusion: Drug Discovery and Development in Industry	801	
Reference	802	
 VII		
PHARMACEUTICAL AND CHEMICAL MEANS TO SOLUBILITY AND FORMULATION PROBLEMS		
 34. Drug Nomenclature		
RAFFAELLA.G. BALOCCO MATTAVELLI, JI-CUI DONG, SOPHIE LASSEUR, A. ROMEO AND SABINE KOPP		
I. Introduction	807	
II. Trade Names and Nonproprietary Names	807	
III. Drug Nomenclature		808
IV. Use and Protection of Nonproprietary Names		819
V. Summary		819
References		820
Annex		820
 35. Web Alert: Using the Internet for Medicinal Chemistry		
DAVID CAVALLA		
I. Introduction		825
II. Blogs		826
III. Wikis		826
IV. Compound Information		827
V. Biological Properties of Compounds		829
VI. Drug Information		831
VII. Physical Chemical Information		832
VIII. Prediction and Calculation of Molecular Properties		832
IX. Chemical Suppliers		834
X. Chemical Synthesis		835
XI. Chemoinformatics Software Programs		836
XII. Chemical Analysis		836
XIII. Chemical Publications		837
XIV. Patent Information		838
XV. Toxicology		839
XVI. Meta-Sites and Technology Service Provider Databases		841
XVII. Conclusion		842
 36. Protection of Inventions in Medicinal Chemistry		
RICHARD LUTHI AND CHRISTOPHER BRICE		
I. Patents and the Medicinal Chemist		843
II. What Kinds of Medical Inventions can be Patented?		845
III. The Basics of Patent Law		846
IV. The Role of the Medicinal Chemist in the Patent Arena		858
V. Patents as a Source of Scientific Information		860
VI. Other Forms of Protection		861
VII. Conclusion		862
 Index		863

This page intentionally left blank

List of Contributors

- Anne Badel** UMR-S973, MTi, University Paris Diderot, Paris, France
- Patrick Bazzini** PC SAS, Bd Gonthier d'Andernach, Illkirch, France
- Frans M. Belpaire** Heymans Institute for Pharmacology, Gent, Belgium
- Alexandre Borrel** University of Helsinki, Helsinki, Finland; UMR-S973, MTi, University Paris Diderot, Paris, France
- Koen Boussey** Laboratory of Medical Biochemistry and Clinical Analysis, Faculty of Pharmaceutical Sciences, Gent University, Gent, Belgium
- Christopher Brice** Consultant, Luthi & Co., Intellectual Property Law Offices, of Tel Aviv, Israel
- Anne-Claude Camproux** UMR-S973, MTi, University Paris Diderot, Paris, France
- David Cavalla** Numedicus Ltd, Cambridge, England
- Paola Ciapetti** Head of Medicinal Chemistry, Novalyst Discovery, Illkirch, Cedex, France
- Hongming Chen** Chemistry Innovation Centre, Discovery Sciences, AstraZeneca R&D, Mölndal, Sweden
- Yong Mi Choi-Sledeski** Sanofi, Boston, MA, USA
- Bernd Clement** Pharmaceutical Institute, Christian Albrechts-University, Gutenbergstraße, Kiel, Germany
- Gordon M. Cragg** NIH Special Volunteer, Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, United States National Cancer Institute, Frederick, Maryland, USA
- Patrick M. Dansette** Laboratoire de Chimie et Biochimie Pharmacologique et Toxicologique, Université Paris Descartes, Sorbonne Paris Cité, Paris
- Pierre deMontigny** Sanofi, Waltham, MA, U.S.A
- Ji-Cui Dong** INN Programme, World Health Organization, Geneva Essential Medicines and Health Products, Regulation of Medicines and Other Health, Technologies, Technologies Standards and Norms
- Iain G. Dougall** IGD Consultancy Ltd, Loughborough, Leics, UK
- Ola Engkvist** Chemistry Innovation Centre, Discovery Sciences, AstraZeneca R&D, Mölndal, Sweden
- Bernard Faller** Novartis Pharma AG, Werk Klybeck Klybeckstrasse, Basel, Switzerland
- Bennett T. Farmer** Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, USA
- Delphine Flatters** UMR-S973, MTi, University Paris Diderot, Paris, France
- Bruno Galli** Novartis Pharma AG, Lichtstrasse, Basel, Switzerland
- Colette Geneix** UMR-S973, MTi, University Paris Diderot, Paris, France
- Bruno Giethlen** Novartis Pharma AG, Basel, Switzerland
- Serge Grisoni** Pierre Fabre Research Institute, Toulouse, Cédex, France
- Gerhard Gross** Scientific Director at Janssen in Beerse, Belgium
- David Harris** Sanofi, Waltham, MA, U.S.A
- Yuichi Hashimoto** Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan
- Chris Ho** Sanofi, Waltham, MA, U.S.A
- Andrew L. Hopkins** College of Life Sciences, University of Dundee, Dundee, Scotland, UK
- Hiba Abi Hussein** UMR-S973, MTi, University Paris Diderot, Paris, France
- Peter Imming** Institut fuer Pharmazie, Martin-Luther-Universitaet Halle-Wittenberg, Halle, Germany
- Minoru Ishikawa** Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan
- Tim Jonckers** Janssen Research & Development Janssen Infectious Diseases – Diagnostics BVBA, Turnhoutseweg, Beerse, Belgium
- Sabine Kopp** World Health Organization, Geneva Essential Medicines and Health Products, Regulation of Medicines and Other Health, Technologies, Technologies Standards and Norms
- David G.I. Kingston** Department of Chemistry and the Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, Virginia, USA
- Thierry Kogej** Chemistry Innovation Centre, Discovery Sciences, AstraZeneca R&D, Mölndal, Sweden
- Sophie Lasseur** INN Programme, World Health Organization, Geneva Essential Medicines and Health Products, Regulation of Medicines and Other Health, Technologies, Technologies Standards and Norms
- Dominique Lesuisse** Lead Generation Board, Sanofi, AVENUE PIERRE BROSOLETTTE, CHILLY MAZARIN - France
- Harvey Lieberman** Sanofi, Waltham, MA, USA
- Richard Luthi** Founding partner, Luthi & Co., Intellectual Property Law Offices, of Tel Aviv, Israel
- Anne-Christine Macherey** Prévention du Risque Chimique, Gif sur Yvette, France

- André Mann** LAAS-CNRS, Université Louis Pasteur, Strasbourg, France
- Raffaella G. Balocco Mattavelli** INN Programme, World Health Organization, Geneva Essential Medicines and Health Products, Regulation of Medicines and Other Health, Technologies, Technologies Standards and Norms
- Christophe Morice** Prestwick Chemical Inc., Boulevard Gonthier, Illkirch, France
- Richard Morphy** Eli Lilly, Erl Wood Manor, Windlesham, Surrey, UK
- David J. Newman** NIH Special Volunteer, Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, United States National Cancer Institute, Frederick, Maryland, USA
- Anne Olivier** Sanofi-Aventis Recherche et Développement, Chilly-Mazarin, France
- Michel Petitjean** UMR-S973, MTi, University Paris Diderot, Paris, France
- Pierre Raboisson** Janssen Infectious Diseases and Diagnostics BVBA, Beerse, Belgium
- Zoran Rankovic** Eli Lilly, Indianapolis, IN, USA
- Leslie Regad** UMR-S973, MTi, University Paris Diderot, Paris, France
- Allen B. Reitz** Fox Chase Chemical Diversity Center, Inc., Doylestown, PA, USA
- Bernd U. Riebesehl** Technical Research & Development, Novartis Pharma AG, Basel, Switzerland
- Jean-Philippe Rocher** Pierre Fabre Research Institute, Castres, France
- Tiago Rodrigues** Swiss Federal Institute of Technology (ETH), Department of Chemistry and Applied Biosciences, Zürich, Switzerland
- A. Romeo** INN Programme, World Health Organization, Geneva Essential Medicines and Health Products, Regulation of Medicines and Other Health, Technologies, Technologies Standards and Norms
- Jean-Michel Rondeau** Novartis Institutes for BioMedical Research, Basel, Switzerland
- Laurent Schaeffer** Prestwick Chemical Inc., Boulevard Gonthier, Illkirch, France
- Jean-Michel Scherrmann** Department of Pharmaceutical Sciences, University Paris Descartes, Paris, France
- Gisbert Schneider** Swiss Federal Institute of Technology (ETH), Department of Chemistry and Applied Biosciences, Zürich, Switzerland
- Herman Schreuder** Sanofi, R&D LGCR/Struct., Design & Informatics FF, Frankfurt am Main, Germany
- Wolfgang Sippl** Medicinal Chemistry, Institute of Pharmacy, Martin-Luther-Universität Halle-Wittenberg, Halle (Saale), Germany
- Erin M. Skoda** Department of Chemistry, University of Pittsburgh, Pittsburgh, PA, USA
- Olivier Taboureau** UMR-S973, MTi, University Paris Diderot, Paris, France
- Bernard Testa** Department of Pharmacy, Lausanne University Hospital (CHUV), Lausanne, Switzerland
- John Unitt** Sygnature Discovery Limited, BioCity, Nottingham, UK
- Johan Van de Voorde** Vascular Research Unit, Ghent University, Gent, Belgium
- Walter M.M. Van den Broeck** Janssen Infectious Diseases BVBA, Beerse, Belgium
- N. Murti Vemuri** CMC Subject Matter Expert at BARDA/HHS, Conceptual MindWorks, Inc., Flemington, NJ, USA
- Venkata Velvadapu** Fox Chase Chemical Diversity Center, Inc., Doylestown, PA, USA
- Franz Weiberth** Sanofi, Waltham, MA, U.S.A
- Bruno Villoutreix** Université Paris Diderot, Sorbonne Paris Cité, Inserm, Paris, France
- Camille G. Wermuth** Founder of Prestwick Chemical, Strasbourg, France
- Peter Wipf** Department of Chemistry, University of Pittsburgh, Pittsburgh, PA, USA
- Gerhard Wolber** Computer-Aided Molecular Design, Institute of Pharmacy, Freie Universität Berlin, Königin-Luisestr., Berlin, Germany

Foreword

The world's economy depends to a significant extent on our ability to deliver affordable and sustainable healthcare. As such, this new edition of *The Practice of Medicinal Chemistry* plays an important role in educating the next generation of scientists in the area as it goes beyond the simple delivery of new healing drugs to combat disease and illness. It enriches our knowledge and the very understanding of the lives of everyone on the planet. It is truly amazing how the different scientific disciplines can combine in this way to design and make such a wonderful array of functional molecules to serve some of our current needs. Nevertheless, the future presents enormous healthcare challenges that can only be met by appropriate investment and further fundamental scientific discovery. This new edition of *The Practice of Medicinal Chemistry* provides a unique scholarly compilation of the tools, techniques, and methods necessary to begin this journey of discovery, whether in industry or academia.

The book's practical overview differentiates this text from others. It provides a menu of topics that can be consulted individually, while also providing a holistic view covering the history of drug discovery through to the issues of today involving the consumption and production of pharmaceuticals. The process of drug discovery has become a highly complex operation requiring the medicinal chemist to acquire wide-ranging skills from areas such as biology, technology, modelling, delivery, physiochemistry, and synthesis. To pull this together in a single book is a heroic task that these authors have done magnificently.

As our science moves forward toward more biologicals, smaller volume products that are focused on patients, and more sustainable and flexible manufacturing in an ever more regulated environment, we will require new generations of creative individuals. They will need to be ever more innovative, using all the tools our modern society can offer. In particular, big-data mining, tissue sampling and genomic mapping, the "Internet of Things," and wearable health monitors will all be likely components in the armory of the next breed of medicinal chemist.

I regard this expanded textbook as essential reading for all those new to the field. It also provides a quality check for current practitioners in this rapidly evolving environment. The book also does not shy away from providing a future vision of the trends of the discipline. It is written by experts who elegantly convey their passion, experience, and insight for the benefit of all readers.

I therefore welcome this updated and expanded version of *The Practice of Medicinal Chemistry* and believe it provides—as did past editions—the bedrock of our discipline.

Steven V. Ley
Cambridge

<http://www.leygroup.ch.cam.ac.uk/>

This page intentionally left blank

Preface to the Fourth Edition

Bringing a new drug to patients is both a privilege and a challenge fraught with success and failure. A privilege because there can be no greater calling than to alleviate suffering to enable a healthier life.

Without health, life is not life; it is only a state of languor and suffering—an image of death. *Buddha*

A challenge fraught with success and failure as no drug makes it from idea to patients without experiencing success and failure.

Success is not final; failure is not fatal. It is the courage to continue that counts. *Winston Churchill*

Prof. Camille Wermuth recognized the need to capture in a single volume the essence of the disciplines needed by medicinal chemists, so as to enable those just entering the field or the seasoned professional to keep pace with the ever-changing nature of drug discovery and development. His vision became *The Practice of Medicinal Chemistry*, providing the medicinal chemistry community with access to experts from across the industry and academia who would share their knowledge to educate the community, thereby preparing the community to recognize and seize opportunities as they emerged.

Fortune favors the prepared mind. *Louis Pasteur*

The fourth edition has built off the previous editions. It is updated to reflect developments over the last seven years, including five new chapters on topics such as the evaluation of the biological activity of compounds and systems biology. More than seventy experts from ten countries have shared their insights and perspectives on the practice of medicinal chemistry.

The editorial work for the fourth edition has been shared by Camille Wermuth, Pierre Raboisson, Didier Rognan, and Dave Aldous. Odile Blin helped organize and shape how we initiated the fourth edition; we are indebted to her tireless professionalism. The editors wish to express their thanks to Molly M. McLaughlin and the Elsevier Academic Press, who have worked with us to keep this project moving forward.

I believe my final quote—from Jason Calacanis—captures the challenges medicinal chemists face every day.

You have to have a big vision and take very small steps to get there. You have to be humble as you execute but visionary and gigantic in terms of your aspiration. In the Internet industry, it's not about grand innovation; it's about a lot of little innovations—every day, every week, every month—making something a little bit better. *Jason Calacanis*

Medicinal chemistry is a highly collaborative and iterative process that has many paths. Being open, collaborative, and humble are qualities that will help you successfully navigate these paths from idea to patient.

This page intentionally left blank

Preface to the Third Edition

Like the preceding editions of this book, this third edition treats of the essential elements of medicinal chemistry in a unique volume. It provides a practical overview of the daily problems facing medicinal chemists, from the conception of new molecules through to the production of new drugs and their legal/economic implications. This edition has been updated, expanded and refocused to reflect developments in the past 5 years, including 11 new chapters on topics such as hit identification methodologies and cheminformatics. More than 50 experts in the field from eight different countries, who have benefited from years of practical experience, give personal accounts of both traditional methodologies and the newest discovery and development technologies, providing readers with an insight into medicinal chemistry.

A major change in comparison to the previous editions was the decision to alleviate my editorial burden in sharing it with seven section editors, each being responsible for one of the eight sections of the book. I highly appreciated their positive and efficacious collaboration and express them my warmest thanks (in the alphabetical order) to Michael Bowker, Hugo Kubinyi, John Proudfoot, Bryan Reuben, Richard Silverman, David Trigg and Han van de Waterbeemd.

Another change was the decision taken by Elsevier/Academic Press to publish the book in full colors thus rendering it more pleasant and user-friendly. I take this occasion to thank Keri Witman, Pat Gonzales, Kirsten Funk and Renske van Dijk for having successively ensured the editorial development of the book. Taking into account that we had to work with a cohort of about 50 authors, each of them having his personality, his original approach and his main busy professional life, this was not an easy task. I am deeply indebted to my assistant Odile Blin for the way she had mastered, efficiently and with friendliness, all the secretarial work and particularly the contacts with the different authors and with the Elsevier development editors. As for the earlier editions, I also want to express my gratitude to my wife Renée and my daughters Delphine, Joëlle and Séverine for all their encouragements and for sacrificing many hours of family life in order to leave me enough free time to edit this new version of the "Medicinal Chemist's Bible."

My final thoughts go to the future readers of the book, and especially to the newcomers in Medicinal Chemistry having the curiosity to read the preface. I cannot resist giving them some advice for doing good science.

First of all, be open-minded and original. As Schopenhauer noted, the task of the creative mind is "not so much to see what no one has seen yet; but to think what nobody has thought yet, about what everyone sees." A wonderful illustration is found in Peter Hesse's cartoon below.

Second, always keep in mind that the object of Medicinal Chemistry is to synthesize new drugs useful for suffering patients. Like many scientists, medicinal chemists, have to navigate between two tempting reefs. On one side they should avoid doing "NAAR": non-applicable applied research, on the other side they may be attracted by "NFBF": non-fundamental basic search."

Third, convinced as they may be that the neighbors grass is always greener, they may be attracted to start their research in using as a hit a recently published competitor's product. In fact, the published compound may exhibit only a weak activity, therefore be very careful when starting a new program and never forget that the worst thing a medicinal chemist can do is to prepare a me-too of an inactive compound!

Camille G. Wermuth

This page intentionally left blank

Preface to the Second Edition

Like the first edition of *The Practice of Medicinal Chemistry* (nicknamed 'The Bible' by medicinal chemists) the second edition is intended primarily for organic chemists beginning a career in drug research. Furthermore, it is a valuable reference source for academic, as well as industrial, medicinal chemists. The general philosophy of the book is to complete the biological progress – Intellectualization at the level of function using the chemical progress Intellectualization at the level of structure (Professor Samuel J. Danishevsky, *Studies in the chemistry and biology of the epothilones and eleutherobins*, Conference given at the XXXIV^{èmes} Rencontres Internationales de Chimie Thérapeutique, Faculté de Pharmacie, Nantes, 8–10 July, 1998).

The recent results from genomic research have allowed for the identification of a great number of new targets, corresponding to hitherto unknown receptors or to new subtypes of already existing receptors. The massive use of combinatorial chemistry, associated with high throughput screening technologies, has identified thousands of hits for these targets. The present challenge is to develop these hits into usable and useful drug candidates. This book is, therefore, particularly timely as it covers abundantly the subject of drug optimization.

The new edition of the book has been updated, expanded and refocused to reflect developments over the nine years since the first edition was published. Experts in the field have provided personal accounts of both traditional methodologies, and the newest discovery and development technologies, giving us an insight into diverse aspects of medicinal chemistry, usually only gained from years of practical experience.

Like the previous edition, this edition includes a concise introduction covering the definition and history of medicinal chemistry, the measurement of biological activities and the three main phases of drug activity. This is followed by detailed discussions on the discovery of new lead compounds including automated, high throughput screening techniques, combinatorial chemistry and the use of the internet, all of which serve to reduce pre-clinical development times and, thus, the cost of drugs. Further chapters discuss the optimization of lead compounds in terms of potency, selectivity, and safety; the contribution of genomics; molecular biology and X-ray crystallization to drug discovery and development, including the design of peptidomimetic drugs; and the development of drug-delivery systems, including organ targeting and the preparation of pharmaceutically acceptable salts. The final section covers legal and economic aspects of drug discovery and production, including drug sources, good manufacturing practices, drug nomenclature, patent protection, social-economic implications and the future of the pharmaceutical industry.

I am deeply indebted to all co-authors for their cooperation, for the time they spent writing their respective contributions and for their patience during the editing process. I am very grateful to Didier Rognan, Paola Ciapetti, Bruno Giethlen, Annie Marcincal, Marie-Louise Jung, Jean-Marie Contreras and Patrick Bazzini for their helpful comments.

My thanks go also to the editorial staff of *Academic Press* in London, particularly to Margaret Macdonald and Jacqueline Read. Last but not least, I want to express my gratitude to my wife Renée for all her encouragements and for her comprehensiveness.

Camille G. Wermuth

This page intentionally left blank

Preface to the First Edition

The role of chemistry in the manufacture of new drugs, and also of cosmetics and agrochemicals, is essential. It is doubtful, however, whether chemists have been properly trained to design and synthesize new drugs or other bioactive compounds. The majority of medicinal chemists working in the pharmaceutical industry are organic synthetic chemists with little or no background in medicinal chemistry who have to acquire the specific aspects of medicinal chemistry during their early years in the pharmaceutical industry. This book is precisely aimed to be their 'bedside book' at the beginning of their career.

After a concise introduction covering background subject matter, such as the definition and history of medicinal chemistry, the measurement of biological activities and the three main phases of drug activity, the second part of the book discusses the most appropriate approach to *finding a new lead compound or an original working hypothesis*. This most uncertain stage in the development of a new drug is nowadays characterized by high-throughput screening methods, synthesis of combinatorial libraries, data base mining and a return to natural product screening. The core of the book (Parts III to V) considers the *optimization of the lead in terms of potency, selectivity, and safety*. In 'Primary Exploration of Structure-Activity Relationships', the most common operational stratagems are discussed, allowing identification of the portions of the molecule that are important for potency. 'Substituents and functions' deals with the rapid and systematic optimization of the lead compound. 'Spatial Organization, Receptor Mapping and Molecular Modelling' considers the three-dimensional aspects of drug-receptor interactions, giving particular emphasis to the design of peptidomimetic drugs and to the control of the agonist-antagonist transition. Parts VI and VII concentrate on the definition of satisfactory drug-delivery conditions, i.e. means to ensure that the molecule reaches its target organ. Pharmacokinetic properties are improved through adequate chemical modifications, notably prodrug design, obtaining suitable water solubility (of utmost importance in medical practice) and improving organoleptic properties (and thus rendering the drug administration acceptable to the patient). Part VIII, 'Development of New Drugs: Legal and Economic Aspects', constitutes an important area in which chemists are almost wholly self taught following their entry into industry.

This book fills a gap in the available bibliography of medicinal chemistry texts. There is not, to the author-editor's knowledge, any other current work in print which deals with the practical aspects of medicinal chemistry, from conception of molecules to their marketing. In this single volume, all the disparate bits of information which medicinal chemists gather over a career, and generally share by word-of-mouth with their colleagues, but which have never been organized and presented in coherent form in print, are brought together. Traditional approaches are not neglected and are illustrated by modern examples and, conversely, the most recent discovery and development technologies are presented and discussed by specialists. Therefore, *The Practice of Medicinal Chemistry* is exactly the type of book to be recommended as a text or as first reading to a synthetic chemist beginning a career in medicinal chemistry. And, even if primarily aimed at organic chemists entering into pharmaceutical research, all medicinal chemists will derive a great deal from reading the book.

The involvement of a large number of authors presents the risk of a certain lack of cohesiveness and of some overlaps, especially as each chapter is written as an autonomic piece of information. Such a situation was anticipated and accepted, especially for a first edition. It can be defended because each contributor is an expert in his/her field and many of them are 'heavyweights' in medicinal chemistry. In editing the book I have tried to ensure a balanced content and a more-or-less consistent style. However, the temptation to influence the personal views of the authors has been resisted. On the contrary, my objective was to combine a plurality of opinions, and to present and discuss a given topic from different angles. Such as it is, this first edition can still be improved and I am grateful in advance to all colleagues for comments and suggestions for future editions.

Special care has been taken to give complete references and, in general, each compound described has been identified by at least one reference. *For compounds for which no specific literature indication is given, the reader is referred to the Merck Index.*

The cover picture of the book is a reproduction of a copperplate engraving designed for me by the late Charles Gutknecht, who was my secondary school chemistry teacher in Mulhouse. It represents an extract of Brueghel's engraving *The alchemist ruining his family in pursuing his chimera*, surmounted by the aquarius symbol. Represented on the left-hand side is my lucky charm castor oil plant (*Ricinus communis* L., *Euphorbiaceae*), which was the starting point of the pyridazine chemistry in my laboratory. The historical cascade of events was as follows: cracking of castor oil produces n-heptanal and aldolization of n-heptanal – and, more generally, of any enolisable aldehyde or ketone – with pyruvic acid leads to α -hydroxy- γ -ketonic acids. Finally, the condensation of these keto acids with hydrazine yields pyrodazones. Thus, all our present research on pyridazine derivatives originates from my schoolboy chemistry, when I prepared in my home in Mulhouse n-heptanal and undecylenic acid by cracking castor oil!

Preparing this book was a collective adventure and I am most grateful to all authors for their cooperation and for the time and the effort they spent to write their respective contributions. I appreciate also their patience, especially as the editing process took much more time than initially expected.

I am very grateful to Brad Anderson (University of Utah, Salt Lake city), Jean-Jacques André (Marion Merrell Dow, Strasbourg), Richard Baker (Eli Lilly, Erl Wood, UK), Thomas C. Jones (Sandoz, Basle), Isabelle Morin (Servier, Paris), Bryan Reuben (London South Bank University) and John Topliss (University of Michigan, Ann Arbor) for their invaluable assistance, comments and contributions.

My thanks go also to the editorial staff of Academic Press in London, Particularly to Susan Lord, Nicola Linton and Fran Kingston, to the two copy editors Len Cegiela and Peter Cross, and finally, to the two secretaries of our laboratory, François Herth and Marylse Wernert.

Last but not least, I want to thank my wife Renée for all her encouragement and for sacrificing evenings and Saturday family life over the past year and a half, to allow me to sit before my computer for about 2500 hours!

Camille G. Wermuth

GENERAL ASPECTS OF MEDICINAL CHEMISTRY

This page intentionally left blank

Medicinal Chemistry: Definitions and Objectives, Drug Activity Phases, Drug Classification Systems

Peter Imming

Institut fuer Pharmazie, Martin-Luther-Universitaet Halle-Wittenberg, Halle, Germany

OUTLINE

I. Definitions and Objectives	3	C. The Pharmacodynamic Phase	8
A. Medicinal Chemistry and Related Disciplines and Terms	3	D. The Road to Successful Drug Development?	8
B. Drugs and Drug Substances	4	III. Drug Classification Systems	8
C. Stages of Drug Development	5	A. Classification by Target and Mechanism of Action	8
II. Drug Activity Phases	7	B. Other Classification Systems	11
A. The Pharmaceutical Phase	7	References	12
B. The Pharmacokinetic Phase	7		

Medicinal chemistry remains a challenging science which provides profound satisfaction to its practitioners. It intrigues those of us who like to solve problems posed by nature. It verges increasingly on biochemistry and on all the physical, genetic and chemical riddles in animal physiology which bear on medicine. Medicinal chemists have a chance to participate in the fundamentals of prevention, therapy and understanding of diseases and thereby to contribute to a healthier and happier life. **A Burger [1]**

I. DEFINITIONS AND OBJECTIVES

A. Medicinal Chemistry and Related Disciplines and Terms

A definition of medicinal chemistry was given by a IUPAC specialized commission: “*Medicinal chemistry* concerns the discovery, the development, the identification and the interpretation of the mode of action of biologically active compounds at the molecular level. Emphasis is put on drugs, but the interests of the medicinal chemist are not restricted to drugs but include bioactive compounds in general. Medicinal chemistry is also concerned with the study, identification, and synthesis of the metabolic products of these drugs and related compounds” [2].

Drugs—natural and synthetic alike—are chemicals used for medicinal purposes. They interact with complex chemical systems of humans or animals. Medicinal chemistry is concerned with this interaction, focusing on the organic and biochemical reactions of drug substances with their targets. This is one aspect of drug chemistry.

Other important aspects are the synthesis and the analysis of drug substances. The two latter aspects together are sometimes called *pharmaceutical chemistry*, but the synthesis of drugs is considered by some people—mainly chemists—to be part of medicinal chemistry, denoting analytical aspects as pharmaceutical chemistry. In German faculties of pharmacy, the literal translations of pharmaceutical and medicinal chemistry—Pharmazeutische and Medizinische Chemie—are used synonymously.

The general study of drugs is called *pharmacy* or *pharmacology*. A common narrower definition of pharmacology concentrates on the fate and effects of a drug in the body. *Clinical chemistry*, a different subject, is concerned with the determination of physiological and pathophysiological parameters in body fluids, such as enzyme activities and metabolites in blood and urine. The term *biopharmacy* has been reserved for the investigation and control of absorption, distribution, metabolism, excretion, and toxicology (ADMET) of drug substances.

Some further terms are more or less synonymous with medicinal chemistry: (*molecular*) *pharmacochemistry*, *drug design*, *selective toxicity*. The French equivalent to medicinal chemistry is *chimie thérapeutique*, and the German terms are Medizinische/Pharmazeutische Chemie and Arzneimittelforschung.

In academia, medicinal chemistry is a major subject in most pharmacy faculties—both for undergraduates and in research—and in many chemistry faculties. In the pharmaceutical industry, medicinal chemistry is at the heart of the search for new medicines.

The main activities of medicinal chemists are evident in the analysis of their most important scientific journals (e.g., *Journal of Medicinal Chemistry*, *European Journal of Medicinal Chemistry*, *Bioorganic and Medicinal Chemistry*, *ChemMedChem*, *Archiv der Pharmazie*, *Arzneimittelforschung*, *Chemical and Pharmaceutical Bulletin*).

The *objectives of medicinal chemistry* are as easily formulated as they are difficult to achieve: find, develop, and improve drug substances that cure or alleviate diseases (see below, [Section I.C.](#)) and understand the causative and accompanying chemical processes (see below, [Section III.A.](#)).

Medicinal chemistry is an interdisciplinary science covering a particularly wide domain situated at the interface of organic chemistry with life sciences such as biochemistry, pharmacology, molecular biology, genetics, immunology, pharmacokinetics, and toxicology on one side, and chemistry-based disciplines such as physical chemistry, crystallography, spectroscopy, and computer-based techniques of simulation, data analysis, and data visualization on the other side.

B. Drugs and Drug Substances

Drugs are composed of *drug substances* (syn. *active pharmaceutical ingredients*, APIs) and *excipients* (syn. *ancillary substances*). The combination of both is the work of pharmaceutical technology (syn. *galenics*) and denoted a *formulation*.

In 2014, the World Drug Index contained over 80,000 marketed and development drug substances [3]. The United States *Orange Book* listed approx. 3,500 products in 2014, and the United States Pharmacopeia contains monographs of approx. 1,400 small-molecules Active Pharmaceutical Ingredients (APIs) and 160 biologic drug substances [4]. In 2013 in Germany, the “Rote Liste” contained approximately 6,000 drugs in 7,500 formulations representing approximately 2,000 APIs [5]. The WHO Essential Medicines List held approximately 350 drug substances in 2013 that WHO claims sufficient for the treatment of approx. 90 percent of all diseases where drugs are useful [6].

What makes a chemical “drug-like?” Because of the versatility of their molecular targets (see below), there can be no universal characteristic of drug substances. However, since the general structure of the target organisms is identical, generalizations as to drug substance structure are possible for biopharmacy [7,8]. For a chemical to be readily absorbed by the gut and distributed in the body, its size, hydrophilicity/lipophilicity ratio, stability toward acid media and hydrolytical enzymes, etc. have to meet defined physicochemical criteria. A careful analysis of reasons for drug attrition revealed that only 5 percent were caused by pharmacokinetic difficulties, whereas 46 percent were due to insufficient efficacy and 33 percent to adverse reactions in animals or humans [9]. Since both wanted and unwanted effects are due to the biological activity, 79 percent of drug candidates had unpredicted or wrongly predicted sum activities.

Predictions of toxicity from molecular features are still precarious [10–12]. Only rather general rules are for sure; such as avoidance of very reactive functional groups, for example, aldehyde because of oxidative instability and haptene nature; α,β -unsaturated carbonyl compounds and 2-halopyridines because of their unspecific reactivity as electrophiles. Torcetrapib is a typical example of toxicity—or adverse effects—challenges. It was an anti-atherosclerotic drug candidate promising to become a blockbuster when in latter phase III of clinical trials, an increased risk of mortality led the company to discontinue its development. It was not clear whether the effects

were caused by the mechanism of action—inhibition of cholesteryl ester transfer protein—some other effect or an interaction with another drug. This is just one instance that “it isn’t that simple [and] nothing’s obvious and nothing’s for certain” in rational drug development [13].

C. Stages of Drug Development

Most drugs were discovered rather than developed [14]. That is why a large number of drug substances are natural products or derivatives thereof. It is a matter of debate if ethnic medicines or nature still hold gems as yet undiscovered by pharmacy [15,16]. Synthetic substance collections (“libraries”) have been created through (automated) organic chemistry. The very high number and diversity of natural and synthetic chemical entities is faced with an equally growing number of potential reaction partners (targets) from biochemical and pathophysiological research.

In virtual, biochemical and cell-based testing, compound selections are run against an isolated or physiologically embedded target that may be involved in the disease process [17]. Compounds that exceed a certain threshold value in binding to the target or modulation of some functional signal behind it, are called *hits*. If the identity and purity of the compound and the assay result are confirmed in a multipoint activity determination, the compound rises to the status of *validated hit*. From this one hopes to develop leads. A *lead* is a compound or series of compounds with proven activity and selectivity in a screen and fulfills some drug development criteria such as originality, patentability, and accessibility (by extraction or synthesis). Molecular variation hopefully tunes the physicochemical parameters so that it becomes suitable for ADME. An example of a small optimization algorithm is shown in Figure 1.1.

If the resulting *optimized lead* (*preclinical candidate*) displays no toxicity in cell and animal models, it becomes a *clinical candidate*. If this stands the tests of efficacy and safety in humans and overcomes marketing hurdles, a *new drug entity* will enter the treasure trove of pharmacy. Box 1.1 illustrates that activity is a necessary but not sufficient quality of medicines. There is, of course, no ideal drug in the real world, but one has to find a relative optimum. This often means developing a drug that has a different side-effect profile than drugs marketed for the same therapeutic indication so prescriptions can be tailored to the ways different patients react to a drug.

The role of medicinal chemistry is most prominent in steps one and two of drug development:

1. The discovery step, consisting of the choice of the therapeutic target (biochemical, cellular, or *in vivo* model; see below) and the identification or discovery and production of new active substances interacting with the selected target.
2. The optimization step that deals with the improvement of an active compound. The optimization process primarily takes into account the increase in potency, selectivity, and decrease in toxicity. Its characteristics are the establishment of structure–activity relationships, ideally based on an understanding of the molecular mode of action.

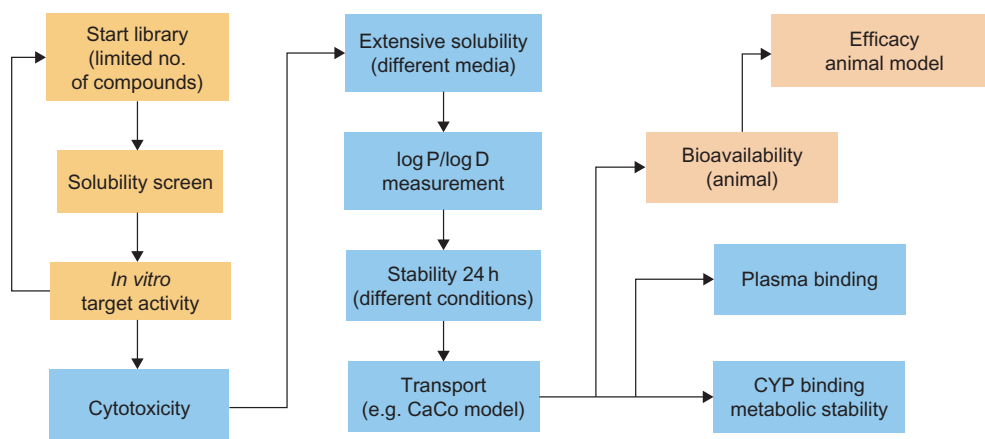


FIGURE 1.1 Example of an optimization algorithm. Source: Adapted from a presentation by Dr. U. Heiser, Probiobdrug AG, Halle, Germany, reproduced with permission.

3. The formulation step, whose purpose is the continuation of the improvement of the pharmacokinetic properties and the fine-tuning of the pharmaceutic properties of active substances to render them suitable for clinical use. This can consist—to name just a few instances—of the preparation of better absorbed compounds, of sustained release formulations, and of water-soluble derivatives or in the elimination of properties related to the patient's compliance (irritation, painful injection, undesirable organoleptic properties). For an example, see [Figure 1.2](#).

The main tasks of medicinal chemistry consist of the optimization of the following characteristics:

- a. Higher affinity and target-intrinsic activation for better clinical activity so the dosage and nonspecific side effects will be as low as possible. There are no examples of drugs that are dosed below 10 mg/day that cause idiosyncratic adverse drug reactions. For drug substances that have to be given in higher doses—i.e., the majority—medicinal chemistry tries to find active derivatives that will be metabolized in a safe way [18]. This includes assaying for inhibition of or reaction with key enzymes of biotransformation, such as oxidases of the cytochrome type, some of which are highly demanded by food constituents and xenobiotics including drug substances [19]. Medicinal chemistry tries to prepare drugs that are not metabolized by bottleneck enzymic pathways [20].
- b. Better selectivity, which may lead to a reduction of unwanted side effects. This sometimes entails the assaying of a very high number of other targets; for example, an antidepressive serotonin re-uptake inhibitor has to be tested against all subtypes of serotonin, adrenaline, and dopamine receptors, plus many other key receptors and enzymes.

BOX 1.1

THE IDEAL NEW DRUG SUBSTANCE

- New chemical entity for patentability and registration.
- Maximum four-step synthesis with, for example, no heavy metal catalysts and no environmentally problematic waste; no chromatographic purification steps; purity >99 percent.
- Stable up to 70°C even in humid air and light.
- Solid-state properties (crystalline, not polymorphous, not hygroscopic) that make it a perfect partner for (tablet) compaction.
- Solubility in water sufficient for the production of stable blood-isotonic solutions.
- Oral bioavailability .90 percent with no interindividual variation.
- Very high activity and pharmacokinetic profile enable once-a-day-dosage at 5–10 mg.

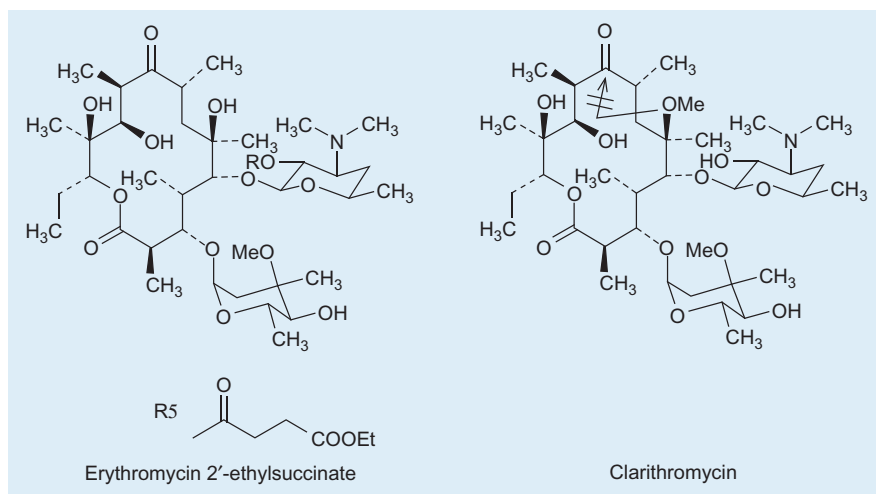


FIGURE 1.2 An example of fine-tuning of pharmacologically active chemicals: Erythromycin 29-ethylsuccinate and clarithromycin are semisynthetic derivatives of the macrolide anti-infective erythromycin. The small molecular change in the former leads to the elimination of bitterness which is important as this class of drugs is often used in pediatrics and administered as a syrup. In the latter, because hemiketal formation is no longer possible (arrow), clarithromycin is stable in the acidic milieu of the stomach (pH 2).

In spite of the high number of compounds, targets, and assays, the development pipeline of new chemical entities as drug substances has not got fuller in the past 20 years. For possible explanations, see the discussion of drug targets below and Ref. [9].

II. DRUG ACTIVITY PHASES

The progression of a drug into the body, to its target(s), and out again can be broken down into three mechanistically distinct phases, the second and third being partly simultaneous. During drug development, all three phases are investigated interdependently, because structural changes required for one phase must not abolish suitability in another phase.

A. The Pharmaceutical Phase

Drug substances are applied orally (preferred mode) or parenterally (e.g., by subcutaneous or intravenous injection, rectally, or through inhalation). A combination of the skills of medicinal chemists and pharmaceutical technologists has to provide the drug candidate in suitable formulations. For tablets, the drug substance needs to be crystalline and not have a low melting point. For injections, it should be water soluble (e.g., as a salt). The required structural features must be compatible with the pharmacological activity, of course.

B. The Pharmacokinetic Phase

For this phase, medicinal chemists and biopharmacists work together to design a compound that will have suitable ADME parameters. Sufficient solubility in an aqueous medium for absorption and blood transport has to be combined with sufficient lipophilicity for passage through cell membranes. If an active compound is too hydrophilic and at the same time contains a carboxylic acid group, for instance, conversion to a simple ester will facilitate absorption. Once in the blood, unspecific esterases will catalyze hydrolysis to the active carboxylic acid form. Such an ester is an instance of a *prodrug*.

Drug substances should remain active and in the body for a period of time that is neither too short nor too long. For many drugs, a metabolic and/or excretion rate that enables “once a day” dosage is sought. Sometimes this requires the identification of sites in the molecule that will be metabolized quickly with concomitant loss of activity. The vasodilator iloprost, for instance, was developed from the endogeneous mediator prostacyclin that has very short half-life both *in vivo* and on the shelf. Modification of several chemically and metabolically vulnerable positions yielded a stable and active derivative—a highly sophisticated product of synthetic medicinal chemistry (Figure 1.3) [21]. By contrast, sometimes functionality is introduced for the acceleration of biotransformation and excretion. Articaine is a local anesthetic of the anilide type. Systemically, it interferes with heart rate—an unwelcome side effect in dentistry. That is why articaine contains an additional ester group. Once in the blood stream, this will be hydrolyzed quickly to an inactive carboxylic acid (Figure 1.4) [22]. Medicinal chemistry here has come full circle, as anilide local anesthetics were developed from ester anesthetics like procain in order to prolong activity.

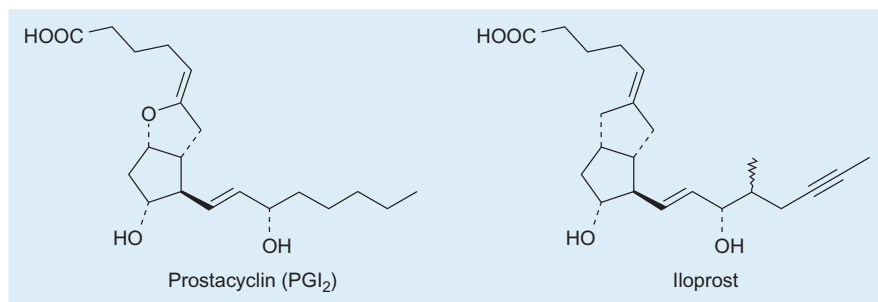


FIGURE 1.3 Prostacyclin and its synthetic analog, iloprost, that combines activity with sufficient *ex vivo* and *in vivo* stability.

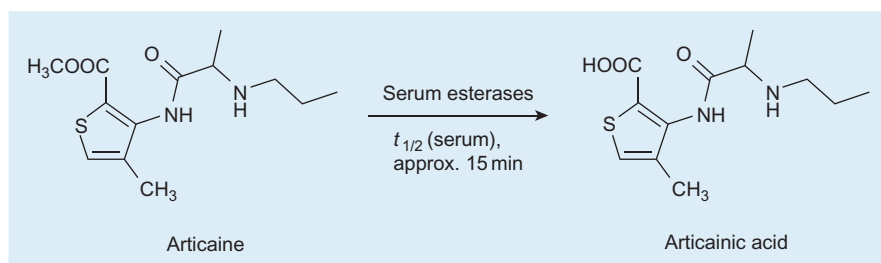


FIGURE 1.4 Articaine, a common local anesthetic dentists use, and its inactive metabolite that is formed off the scene of painful action. The value for $t_{1/2}$ is from the reference Oertel R, Ebert U, Rahn R, Kirch W. The effect of age on pharmacokinetics of the local anesthetic drug articaine. Reg Anesth Pain Med 1999;24:524–8.

C. The Pharmacodynamic Phase

While pharmacokinetics investigates what the body does to the drug, pharmacodynamics is concerned with what the drug does to the body. Most scientists who consider themselves medicinal chemists will be most comfortable with and interested in this phase. They will cooperate with biochemists and pharmacologists to elucidate mechanistic details of the interaction of the drug with its target(s), a topic we will treat in the [Section III](#).

D. The Road to Successful Drug Development?

In the past years, many analyses have appeared that try to explain the dearth of new drug substances in the face of billions of dollars that have been spent, billions of assay data points that have been accumulated, and ten thousands of virtual and thousands of real hits that have been generated. By comparison, the Belgian medicinal chemist Paul Janssen and his relatively small group had tremendous success in the development of new drug entities and activities [23]. It was postulated that the individualization rather than integration of research guidelines into successive hypes (e.g., “as target subtype selective as possible”; “ADME rules have to be strictly adhered to”; “modeling programs automatically give a correct representation of molecules”; “the more combinatorial ligands, the more hits”) is responsible for the disappointing state of drug discovery. What is needed is to keep what we already know about how successful drugs were actually discovered or invented [24], while providing an atmosphere of creativity in a team of scientists from various disciplines. Summarizing their long-lasting experiences in antibacterial research, an industrial team concluded that for this therapeutic area at least, synthesizing novel chemical structures that interact with and block *established* targets in new ways is a robust strategy [24,25].

So what does the increasing knowledge of targets mean for medicinal chemistry? This subject will be introduced in the following paragraphs and discussed in detail in later chapters.

III. DRUG CLASSIFICATION SYSTEMS

Classification systems help with understanding what a drug actually does at the molecular level (classification by target), and they are indispensable for categorizing the large number of drug substances (classification by clinical effect).

A. Classification by Target and Mechanism of Action

1. Targets

Targets are molecular structures, chemically definable by at least a molecular mass, that will undergo a specific interaction with chemicals that we call drugs because they are administered to treat or diagnose a disease [26]. To be meaningful, the interaction has to have a connection with the clinical effect(s). It is very challenging to prove that the interaction of a drug substance with a specific molecular target indeed triggers the clinical effect(s).

A clinically relevant target might consist not of a single biochemical entity but the simultaneous interference of a number of receptors. Only this multi-target interaction will give a net clinical effect that might be considered beneficial. It is only by chance that the current *in vitro* screening techniques will identify drugs that work through such targets.

The number of targets presently used is still open to discussion in medicinal chemistry, but various approaches concurred in finding several hundred. The number of potential targets, however, was estimated to be

several hundred thousand in view of the manifold protein complexes, splicing variants, and possible interventions with signaling pathways [26,27]. The problem with counting is two-fold: first, the identification of the reaction partners of drug substances in the body; and second, exactly what to define and count as the target. A target definition derived from the net effect rather than the direct chemical interaction will require input from systems biology, a research field that promises to affect the drug discovery process significantly [28]. At the other end of the scale of precision, we can define some targets very precisely on the molecular level. For example, we can say that dihydropyridines block the CaV1.2a splicing variant in heart muscle cells of L-type high-voltage activated calcium channels.

The actual depth of detail used to define the target is primarily dependent on the amount of knowledge available about the target and its interactions with a drug. Even if the target structure has already been determined, the molecular effect of the drug could still not be fully described by the interactions with one target protein alone. For example, antibacterial oxazolidinones interact with 23S-rRNA, tRNA, and two polypeptides, ultimately leading to inhibition of protein synthesis [29]. In this case, a description of the mechanism of action that only includes interactions with the 23S-rRNA target would be too narrowly defined. In particular, in situations in which the dynamic actions of the drug substance stimulate or inhibit a biological process, it is necessary to move away from the description of single proteins, receptors and other targets to view the entire signal chain as the target. and so on to view the entire signal chain as the target.

Lists that classify all marketed drug substances according to target, with references, were published. An excerpt is given in Table 1.1 [26].

2. Mechanisms of Action

An effective drug target comprises a biochemical system rather than a single molecule. Present target definitions are static. We know this to be insufficient, but techniques to observe the dynamics of drug–target interactions are just being created. Most importantly, we are not able to gauge the interaction of the biochemical “ripples” that follow the drug’s initial molecular effect. The first molecular step of drug activity consists in mass-action governed drug–target recognition. For clinically observable activity, a series of biochemical steps need to follow that have to shift physiological equilibria in a transient way. Indeed, the gap between chain and circles of molecular events and clinical effects is still wide open, as reflected by the complementarity of target and phenotypic-oriented drug discovery approaches [30].

Although the term “mechanism of action” itself implies a classification according to the dynamics of drug substance effects at the molecular level, the dynamics of these interactions are only speculative models at present, and so mechanism of action can currently only be used to describe static targets, as discussed above.

All drugs somehow interfere with signal transduction, receptor signaling, and biochemical equilibria. For many drugs we know—and for most we suspect—that they interact with more than one target. So there will be simultaneous changes in several biochemical signals, and there will be feedback reactions of the pathways disturbed. In most cases, the net result will not be linearly deducible from single effects. For drug combinations, this is even more complicated. Awareness is also increasing of the nonlinear correlation of molecular interactions and clinical effects. For example, the importance of receptor–receptor interactions (receptor mosaics) was summarized for G-protein-coupled receptors (GPCRs), resulting in the hypothesis that cooperativity is important for the decoding of signals, including drug signals [31]. Table 1.2 lists examples of dynamic molecular mechanisms of drugs. Table 1.1 is the excerpt of an attempt at a complete list of drug targets. Notably, inhibitors and antagonists by far outnumber effectors, agonists, and substitutes. It appears that reconstitution of biochemical and pharmacological balances is more easily achieved by blocking excessive or complementary pathways rather than by substitution or repair of deficient or defective biochemical input.

Greater knowledge of how drugs interact with the body (e.g., mechanisms of action, drug–target interactions) has led to a reduction of established drug doses and inspired the development of newer, highly specific drug substances with a known mechanism of action. However, a preoccupation with the molecular details has resulted in a tendency to focus only on this one aspect of the drug effects. For example, cumulative evidence suggests that the proven influence of certain psychopharmaceuticals on neurotransmitter metabolism has little to do with the treatment of schizophrenia or the effectiveness of the drug for this indication [32]. With all our efforts to understand the molecular basis of drug action, we must not fall into the trap of reductionism. For antibacterial research, multitargeting is now considered to be essential [33]. More generally, in recent years the limits of the reductionist approach in drug discovery have become painfully clear. Nobel laureate Roald Hoffmann put it this way: “Chemistry reduced to its simplest terms, is not physics. Medicine is not chemistry ... knowledge of the specific physiological and eventually molecular sequence of events does not help us understand what [a] poet has to say to us” [34]. The cartoon (Figure 1.5) illustrates this point. Although it is too early for systems biology

TABLE 1.1 The Main Drug Target Classes with Examples of Targets and Ligands. A Full List Can Be Found in Ref. [26]

Target class	Target subclass	Target example	Drug substance example (activity)
Enzymes	Oxidoreductases	Aldehyde dehydrogenase	Disulfiram (inhibitor)
	Transferases	Protein kinase C	Miltefosine (inhibitor)
	Hydrolases	Bacterial serine protease	β -Lactams (inhibitors)
	Lyases	DOPA decarboxylase	Carbidopa (inhibitor)
	Isomerases	Alanine racemase	D-cycloserine (inhibitor)
	Ligases (syn. synthases)	Dihydropteroate synthase	Sulphonamides (inhibitors)
Proteins	Growth factors	Vascular endothelial growth factor	Bevacizumab (antibody)
	Immunoglobulins	CD3	Muromonab-CD3 (antibody)
	Integrins	$\alpha 4$ -Integrin subunit	Natalizumab (antibody)
	Tubulin	Human spindle	Vinca alkaloids (development inhibitors)
Substrates, metabolites	Substrate	Asparagine	Asparaginase (enhanced degradation)
	Metabolite	Urate	Rasburicase (enhanced degradation)
Receptors	Direct ligand-gated ion channel receptors	γ -Aminobutyric-acid (GABA)-	Barbiturates (allosteric agonists)
	G-protein-coupled receptors	Acetylcholine receptors	Pilocarpine (muscarinic receptor agonist)
		Opioid receptors	
		Prostanoid receptors	Buprenorphine (κ -opioid antagonist)
			Misoprostol (agonist)
	Cytokine receptors	TNF α receptors	Etanercept (receptor mimic)
	Integrin receptors	Glycoprotein IIb/IIIa receptor	Tirofiban (antagonist)
	Receptors associated with a tyrosine kinase	Insulin receptor	Insulin (agonist)
	Nuclear receptors, steroid hormone receptors	Mineralocorticoid receptor	Aldosterone (agonist)
Ion channels	Nuclear receptors, other	Retinoic acid receptors	Isotretinoin (RAR α agonist)
	Voltage-gated Ca ²⁺ channels	L-type channels	Dihydropyridines (inhibitors)
		K ⁺ channels	Diazoxide (opener)
		Na ⁺ channels	Carbamazepine (inhibitor)
	Ryanodine-inositol 1,4,5-triphosphate receptor Ca ²⁺ channel	Ryanodine receptors	Dantrolene (inhibitor)
	Transient receptor potential Ca ²⁺ channel	TRPV1 receptors	Acetaminophen metabolite (inhibitor)
	Chloride channels	Mast cell chloride channels	Cromolyn sodium (inhibitor)
	Cation-chloride cotransporter family	Thiazide-sensitive NaCl symporter	Thiazide diuretics (inhibitors)
Transport proteins	Na ⁺ /H ⁺ antiporters		Amiloride (inhibitor)
	Proton pumps	H ⁺ /K ⁺ ATPase	Omeprazole (inhibitor)
	Eukaryotic (putative) sterol transporter (EST) family	Niemann-Pick C1 like 1 protein	Ezetimibe (inhibitor)
	Neurotransmitter/Na ⁺ symporter family	Serotonin/Na ⁺ symporter	Paroxetine (inhibitor)

(Continued)

I. GENERAL ASPECTS OF MEDICINAL CHEMISTRY

TABLE 1.1 (Continued)

Target class	Target subclass	Target example	Drug substance example (activity)
DNA, RNA	Nucleic acids	Bacterial 16S-RNA	Aminoglycosides (protein synthesis inhibition)
	Ribosome	Bacterial 30S subunit	Tetracyclines (protein synthesis inhibition)
Physicochemical mechanism	Ion exchange	Hydroxide	Fluoride (enhanced acid stability of adamantine)
	Acid binding	In stomach	Hydrotalcite
	Adsorptive	In gut	Charcoal
	Surface-active	On oral mucosa	Chlorhexidine (disinfectant)
	Oxidative	On skin	Permanganate (disinfectant)
	Reductive	Disulphide bonds	<i>N</i> -acetylcysteine (mucolytic)
	Osmotically active	In gut	Lactulose (laxative)

TABLE 1.2 Examples of Dynamic (Process) Mechanisms of Drug Action

Dynamic mechanism	Example
Covalent modifications of the active center	Acylation of bacterial transpeptidases by β -lactam antibiotics
Drugs that require the receptor to adopt a certain conformation for binding and inhibition	Trapping of K^+ channels by methanesulphonanilide antiarrhythmic agents
Drugs that exert their effect indirectly and require a functional background	The catechol <i>O</i> -methyltransferase inhibitor entacapone, the effect of which is due to the accumulation of nonmetabolized dopamine
Anti-infectives that require the target organism to be in an active, growing state	β -Lactam antibacterials
Molecules requiring activation (prodrugs)	Enalaprilate, paracetamol
Modifications of a substrate or cofactor	Asparaginase, which depletes tumour cells of asparagine; ^a isoniazide, which is activated by mycobacteria leading to an inactive covalently modified NADH ^b
Simultaneous modulation of several signaling systems	GPCR receptor mosaics for the decoding of drug signals
Fluctuations of physiological signaling molecules	Dopamine fluctuations after administration of cocaine, followed by a gradual increase in steady state dopamine concentration ^c

^aGraham ML. Pegaspargase: a review of clinical studies. *Adv Drug Deliv Rev* 2003;55:1293–302.

^bLarsen MH, Vilch  ze C, Kremer L, Besra GS, Parsons L, Salfinger M, et al. Overexpression of *inhA*, but not *kasA*, confers resistance to isoniazid and ethionamide in *Mycobacterium smegmatis*, *M. bovis* BCG, and *M. tuberculosis*. *Mol Microbiol* 2002;46:453–66.

^cHeien ML, Khan AS, Ariansen JL, Cheer JF, Phillips PE, Wassum KM, et al. Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. *Proc Natl Acad Sci USA* 2005;102:10023–8.

to provide clear-cut protocols for medicinal chemistry, translational medicine [35] and other integrative research efforts stress the functional as opposed to reductionist character of living systems, hopefully improving the success rate of drug research [36].

B. Other Classification Systems

From a pharmaceutical standpoint, many different criteria can be used to classify medications: type of formulation, the frequency with which it is prescribed or recommended, price, refundability, prescription or nonprescription medication, etc. If a classification of the APIs is undertaken, numerous possibilities are revealed as well. At the end of the 19th century, drug substances were classified the same as other chemical entities: by nature of their primary elements, functional moieties, or organic substance class. Recently, the idea of classifying drug substances strictly according to their chemical constitution or structure has been revived. Databases attempt to gather

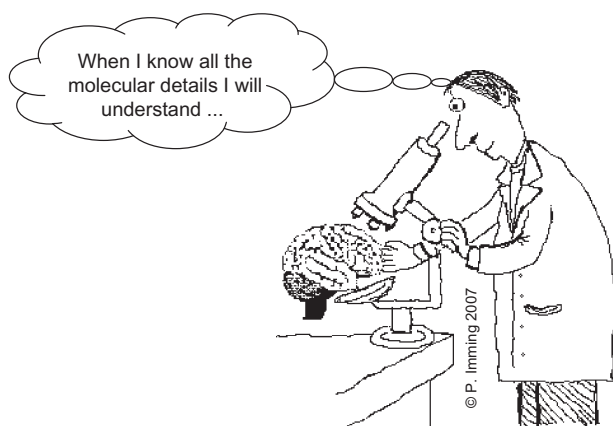


FIGURE 1.5 Searching for molecular mechanisms ... “The meaning of the message will not be found in the chemistry of the ink.” Sperry R. Brain circuits and functions of the mind. Cambridge: Cambridge University Press; 1990. Source: Roger Sperry, neurophysiologist, Nobel Prize in Medicine, 1981.

and organize information on existing or potential drug substances according to their chemical structure and diversity. The objective is to create—virtual or real—substance or fragment “libraries” that contain pertinent information about possible ligands for new targets (e.g., an enzyme or receptor) of clinical interest [37,38], and, more importantly, to understand the systematics of molecular recognition (ligand–receptor) [39,40].

The most commonly used classification system for drug substances is the *ATC system* [41]. It was introduced in 1976 by the Nordic Council on Medicines as a method for carrying out drug utilization studies throughout Scandinavia. In 1981, the World Health Organization recommended the use of the ATC classification for all global drug utilization studies, and in 1982 founded the WHO Collaborating Centre for Drugs Statistics Methodology in Oslo to establish and develop the method. The ATC system categorizes drug substances at five different levels according to (1) the organ or system on which they act (anatomy), (2) therapeutic and pharmacological properties, and (3) chemical properties. The first level comprises the main anatomical groups, while the second level contains the pharmacologically relevant therapeutic subgroup. The third level consists of the pharmacological subgroup, and the fourth the chemical subgroup. The fifth level represents the chemical substance (the actual drug entity). Drugs with multiple effects and different target organs can be found more than once within the system. The anti-inflammatory agent diclofenac, for instance, has three ATC numbers, one of them being M01AB05. This key breaks down to: M01 (musculo-skeletal system; anti-inflammatory and antirheumatic agents, nonsteroids); M01AB (acetic acid derivatives and related substances); and 05 (diclofenac in M01AB). The two other keys classify diclofenac as a topical agent and its use for inflammation of sensory organs.

While ATC is better suited if the emphasis is on therapeutic use, the TCAT system [26,42] puts the target chemistry first, particularly suiting the medicinal chemical approach.

References

- [1] Burger A. Preface. In: Hansch C, Sammes PG, Taylor JB, editors. *Comprehensive medicinal chemistry*. Oxford: Pergamon Press; 1990. p. 1.
- [2] Wermuth CG, Ganellin CR, Lindberg P, Mitscher LA. Glossary of terms used in medicinal chemistry. IUPAC Recommendations 1997, Part I. In: Adam J, editor. *Annual reports in medicinal chemistry*. San Diego, CA: Academic Press; 1998. p. 385–95. For Part II, see: <<http://www.iupac.org/home/publications/provisional-recommendations/published/published-container/glossary-of-terms-used-in-medicinal-chemistry-part-ii.html>>.
- [3] World Drug Index. Available from Thomson Scientific at: <<http://www.thomsonreuters.com/world-drug-index/>>.
- [4] US Pharmacopeial Convention. Available from: <<http://www.usp.org/>>.
- [5] Rote Liste 2013. Frankfurt/Main: Rote Liste Service GmbH. <<http://www.rote-liste.de/>>; 2014.
- [6] World Health Organisation. WHO model list of essential medicines. Available from: <<http://www.who.int/medicines/publications/essentialmedicines/en/>>; 2013.
- [7] Alqahtani S, Mohamed LA, Kaddoumi A. Experimental models for predicting drug absorption and metabolism. *Expert Opin Drug Metab Toxicol* 2013;9:1241–54.
- [8] Cheng F, Li W, Liu G, Tang Y. In silico ADMET prediction: recent advances, current challenges and future trends. *Curr Top Med Chem* 2013;13:1273–89.
- [9] Schuster D, Laggner C, Langer T. Why drugs fail—a study on side effects in new chemical entities. *Curr Pharmaceut Des* 2005;11:3545–59.

- [10] Guengerich FP, MacDonald JS. Applying mechanisms of chemical toxicity to predict drug safety. *Chem Res Toxicol* 2007;20:344–69.
- [11] Bai JP, Abernethy DR. Systems pharmacology to predict drug toxicity: integration across levels of biological organization. *Annu Rev Pharmacol Toxicol* 2012;53:451–73.
- [12] Segall MD, Barber C. Addressing toxicity risk when designing and selecting compounds in early drug discovery. *Drug Discov Today* 2014;19:688–93. Available from: <http://dx.doi.org/10.1016/j.drudis.2014.01.006>.
- [13] Lowe D. In the pipeline: trocetarapib. *Chemistry World* 2007;4:16.
- [14] Sneader W. *Drug discovery*. Chichester: Wiley-Interscience; 2008.
- [15] Li JW, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? *Science* 2009;325:161–5.
- [16] Koehn FE, Carter GT. Industrial natural product chemistry for drug discovery and development. *Nat Prod Rep* 2014;31:35–60.
- [17] Potytrailo R, Rajan K, Stoewe K, Takeuchi I, Chisholm B, Lam H. Combinatorial and high-throughput screening of materials libraries: review of state of the art. *ACS Comb Sci* 2011;13:579–633.
- [18] Kalgutkar AS, Soglia JR. Minimizing the potential for metabolic activation in drug discovery. *Expert Opin Drug Metab Toxicol* 2005;1:91–141.
- [19] Zlokarnik G, Grootenhuys PDJ, Watson JB. High throughput P450 inhibition screen in early drug discovery. *Drug Discov Today* 2005;10:1443–50.
- [20] Pelkonen O, Raunio H. In vitro screening of drug metabolism during drug development: can we trust the predictions? *Exp Opin Drug Metab Toxicol* 2005;1:49–59.
- [21] Skuballa W, Schillinger E, Stuerzebecher CS, Vorbrueggen H. Prostaglandin analogs. Part 9. Synthesis of a new chemically and metabolically stable prostacyclin analog with high and long-lasting oral activity. *J Med Chem* 1986;29:313–15.
- [22] Oertel R, Rahn R, Kirch W. Clinical pharmacokinetics of articaine. *Clin Pharmacokin* 1997;33:417–25.
- [23] Black J. A personal perspective on Dr. Paul Janssen. *J Med Chem* 2005;48:1687–8.
- [24] Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat Rev Drug Discov* 2007;6:29–40.
- [25] Jordan AM, Roughley SD. Drug discovery chemistry: a primer for the non-specialist. *Drug Discov Today* 2009;14:731–44.
- [26] Imming P, Sinning C, Meyer A. Drugs, their targets and the nature and number of drug targets. *Nat Rev Drug Discov* 2006;5:821–34.
- [27] DrugBank. Available from: <http://www.drugbank.ca/>.
- [28] Lowe JA, Jones P, Wilson DM. Network biology as a new approach to drug discovery. *Curr Opin Drug Discov Devel* 2010;13:524–6.
- [29] Colca JR, McDonald WG, Waldon DJ, Thomasco LM, Gadwood RC, Lund ET, et al. Cross-linking in the living cell locates the site of action of oxazolidinone antibiotics. *J Biol Chem* 2003;278:21972–9.
- [30] Swinney DC. Phenotypic vs. target-based drug discovery for first-in-class medicines. *Clin Pharmacol Ther* 2013;93:299–301.
- [31] Fuxe K, Marcellino D, Borroto-Escuela DO, Frankowska M, Ferraro L, Guidolin D, et al. The changing world of G protein-coupled receptors: from monomers to dimers and receptor mosaics with allosteric receptor-receptor interactions. *J Recept Signal Transduct Res* 2010;30:272–83.
- [32] Hyman SE, Fenton WS. What are the right targets for psychopharmacology? *Science* 2003;299:350–1.
- [33] Silver LL. Multi-targeting by monotherapeutic antibacterials. *Nat Rev Drug Discov* 2007;6:41–55.
- [34] Hoffmann R. Speech at the Nobel banquet. Available from: http://nobelprize.org/nobel_prizes/chemistry/laureates/1981/hoffmann-speech.html; 1981.
- [35] Albani S, Prakken B. The advancement of translational medicine-from regional challenges to global solutions. *Nat Med* 2009;15:1006–9.
- [36] McGonigle P, Ruggeri B. Animal models of human disease: challenges in enabling translation. *Biochem Pharmacol* 2014;87:162–71.
- [37] Peng Z. Very large virtual compound spaces: construction, storage and utility in drug discovery. *Drug Discov Today Technol* 2013;10:e387–94.
- [38] Wilde F, Link A. Advances in the design of a multipurpose fragment screening library. *Expert Opin Drug Discov* 2013;8:597–606.
- [39] Hendlich M, Bergner A, Gunther J, Klebe G. Relibase: design and development of a database for comprehensive analysis of protein–ligand interactions. *J Mol Biol* 2003;326:607–20. Available from: <http://relibase.ccdc.cam.ac.uk/>.
- [40] Parenti MD, Rastelli G. Advances and applications of binding affinity prediction methods in drug discovery. *Biotechnol Adv* 2012;30:244–50.
- [41] WHO Collaborating Centre for Drug Statistics Methodology. ATC/DDD Index. Available from: http://www.whocc.no/atc_ddd_index/; 2015.
- [42] Imming P, Buss T, Dailey LA, Meyer A, Morck H, Ramadan M, et al. A classification of drug substances according to their mechanism of action. *Pharmazie* 2004;59:579–89.

Molecular Variations Based on Isosteric Replacements

Paola Ciapetti¹ and Bruno Giethlen²

¹Head of Medicinal Chemistry, Novalyst Discovery, Illkirch, Cedex, France; ²Novartis Pharma AG, Basel, Switzerland

OUTLINE

I. Introduction	181	F. Reversal of Functional Groups	218
II. History: Development of the Isosterism Concept	182	IV. Scaffold Hopping	220
A. The Molecular Number	182	A. Successful Examples of Serendipitous Scaffold Hopping	221
B. The Isosterism Concept	184	B. Scaffold Hopping and Virtual Screening	222
C. The Notion of Pseudoatoms and Grimm's Hydride Displacement Law	184	V. Analysis of the Modifications Resulting from Isosterism	224
D. Erlenmeyer's Expansion of the Isosterism Concept	185	A. Structural Parameters	225
E. Isosterism Criteria: Present Conceptions	185	B. Electronic Parameters	226
F. The Bioisosterism Concept: Friedman's and Thorner's Definitions	186	C. Solubility Parameters	226
III. Currently Encountered Isosteric and Bioisosteric Modifications	186	D. Anomalies in Isosterism	226
A. Replacement of Univalent Atoms or Groups	187	VI. Minor Metalloids-Toxic Isosteres	229
B. Interchange of Divalent Atoms and Groups	188	A. Carbon–Silicon Bioisosterism	229
C. Interchange of Trivalent Atoms and Groups	189	B. Carbon–Boron Isosterism	231
D. Ring Equivalents	189	C. Bioisosteres Involving Selenium	232
E. Groups with Similar Polar Effects: Functional Equivalents	199	References	233

Things are not always what they seem; the first appearance deceives many; the intelligence of a few perceives what has been carefully hidden. . . Anonymous

I. INTRODUCTION

In a biologically active molecule, the replacement of an atom or a group of atoms by another one presenting the same physiochemical properties is based on the concept of isosterism. The notion of isosterism was

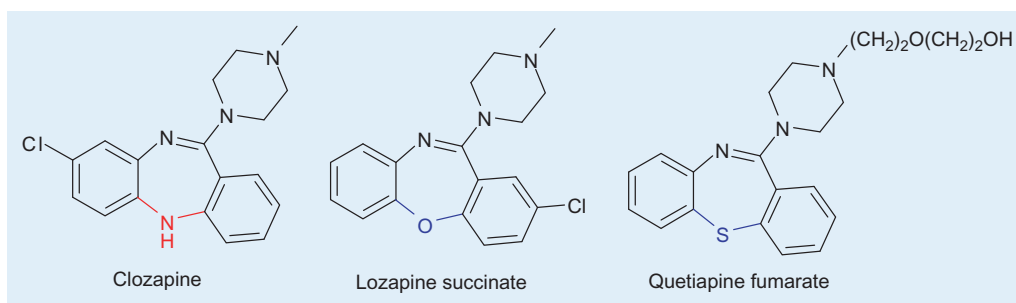


FIGURE 8.1 Isosteric replacements of -NH- with -O- and -S- in clozapine analogs.

introduced in 1919 by Langmuir [1], who was mainly focused on the similarities of electronic and steric arrangement of atoms, groups, radicals, and molecules.

The concept of isosteres was then broadened by Grimm in 1925 with the statement of Hydride Displacement Law, and—further on—Erlenmeyer extended Grimm's classification defining isosteres as atoms, ions, and molecules in which the peripheral layers of electrons can be considered identical. The extensive application of isosterism to modify a part of a biologically active molecule to get another one of similar activity has given rise to the term of "bioisosterism" or nonclassical isosterism. As initially defined by Friedman [2], bioisosteres include all atoms and molecules that fit the broadest definition for isosteres and that elicit the similar biological activity. In medicinal chemistry, the concept of bioisosterism is a research tool of the utmost importance, widely used in analogs design.

The term "analog" is derived from the Greek word *αναλογία* (analogia) and has been used to point out structural and functional similarity. Applied to drugs, an analog of an existing drug shares chemical and therapeutic similarity with the parent compound. This definition implies that three categories of drug analogs can be listed: (a) those presenting chemical and therapeutic similarity; (b) those presenting only similar chemical features; and (c) those eliciting the same pharmacological effect but displaying a completely different chemical scaffold. Isosteres have been classified as either classical or nonclassical according to the degree of electronic and steric likeness. Bioisosterism is used interchangeably with the term nonclassical isosteres. Figure 8.1 reports clozapine and two analogs as an example of classic isosteric replacement of NH with O and S.

An illustration of bioisosteric change is given in Figure 8.2, where the amide moiety of diazepam has been replaced by a triazole ring to give alprazolam.

Taken in its broadest meaning, bioisosterism include the replacement of the initial molecular scaffold by a different one, keeping the same biological activity. This approach, called "scaffold hopping," is well illustrated by molecules like diazepam, zolpidem, zaleplon, and zopiclone, which exert the same biological response acting as full agonists of GABA-A (γ -aminobutyric acid) receptor at the benzodiazepine site though being structurally different (see Figure 8.3).

Isosterism, bioisosterism, and scaffold hopping will be discussed in more details in the following paragraphs.

II. HISTORY: DEVELOPMENT OF THE ISOSTERISM CONCEPT

The development of the concept of isosterism takes its roots in the attempts to extend to whole molecules the knowledge acquired for elements—namely that two elements possessing an identical peripheral electronic distribution do also possess similar chemical properties.

A. The Molecular Number

In 1918 [3], Allen defined the molecular number of a compound in a way similar to the atomic number:

$$N = aN_1 + bN_2 + cN_3 + \dots + zN_i$$

where N = molecular number $N_1, N_2, N_3, \dots, N_i$ = respective atomic numbers of each element of the molecule.

$a, b, \dots, z$ = number of atoms of each element present in the molecule.

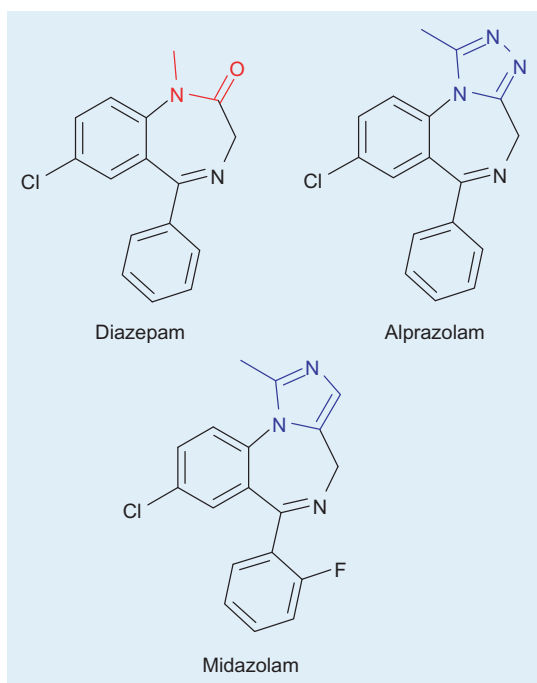


FIGURE 8.2 Example of bioisosteric replacement in the benzodiazepine series.

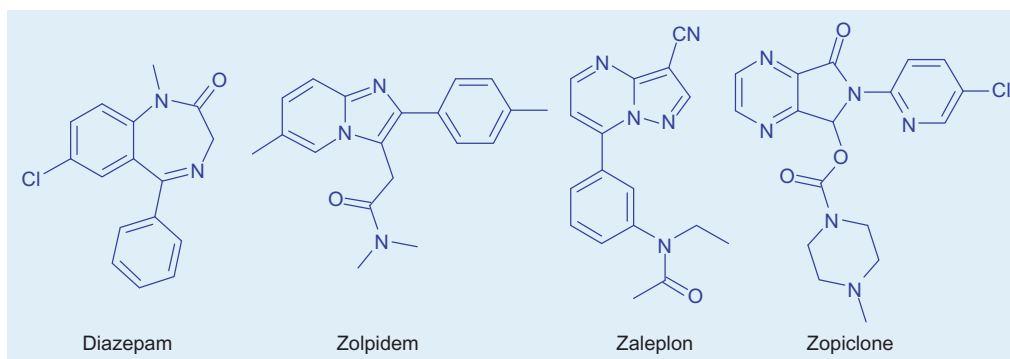


FIGURE 8.3 Scaffold hopping can be considered as the broadest example of bioisosterism: structurally different molecules elicit the same biological activity.

Example: Comparison of the ammonium and sodium cations. The atomic number of nitrogen is 7 and that of hydrogen is 1. Thus, the molecular number of the ammonium cation can be calculated and compared to that of the sodium ion:

	Atomic number		Molecular number
NH_4^+	$7 + (4 \times 1)$	=	11
Na^+	11	=	11

II. LEAD COMPOUND DISCOVERY STRATEGIES

TABLE 8.1 Groups of Isosteres as Identified by Langmuir

Groups	Isosteres
1	H ⁻ , He, Li ⁺
2	O ²⁻ , F ⁻ , Ne, Na ⁺ , Mg ²⁺ , Al ³⁺
3	S ²⁻ , Cl ⁻ , Ar, K ⁺ , Ca ²⁺
↓	↓
8	N ₂ , CO, CN ⁻
9	CH ₄ , NH ₄ ⁺
10	CO ₂ , N ₂ O, N ³⁺ , CNO ⁻
↓	↓
21	SeO ₄ ²⁻ , AsO ₄ ³⁻

Possessing the same molecular number, the ammonium cation should resemble the sodium cation. This is roughly true. More generally, two compounds with identical molecular numbers present at least some similar physical properties (e.g., specific heat).

B. The Isosterism Concept

Independently of Allen, Langmuir in 1919 [4] defined the concept of isosterism:

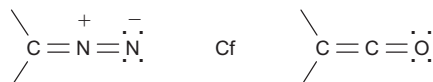
Comolecules are thus isosteric if they contain the same number and arrangement of electrons. The comolecules of isosteres must, therefore, contain the same number of atoms. The essential differences between isosteres are confined to the charges on the nuclei of the constituent atoms.

On the basis of these similarities, Langmuir identified a list of twenty-one groups of isosteres [5]. Some of these are listed in Table 8.1. He further deduced from the octet theory that the number and arrangement of electrons in these molecules are the same. Thus, isosteres were initially defined as those compounds or groups of atoms that have the same number and arrangement of electrons. Then, he defined other relationships in a similar manner. Argon was viewed as an isostere of K⁺ ion and methane as an isostere of NH₄⁺ ion. He deduced that K⁺ ions and NH₄⁺ ions must be similar because argon and methane are very similar in physical properties. The biological similarity of molecules such as CO₂ and N₂O was later coincidentally acknowledged, as both compounds were capable of acting as reversible anesthetics to the slime mold *Physarum polycephalum* [6].

The first example clearly demonstrates that isosterism does not inevitably imply “isoelectric” structures (having the same total electric charge), but it becomes evident that isoelectronic isosteres show the closest analogies:



In the field of organic chemistry, Langmuir predicted the analogy between diazomethane and ketene, which was only discovered later.



C. The Notion of Pseudoatoms and Grimm's Hydride Displacement Law

Later on, in 1925, Grimm [7] formulated the “hydride displacement law,” according to which the addition of hydrogen to an atom confers on an aggregate the properties of the atom of next highest atomic number. An isoelectronic relationship [8] exists among such aggregates, which were named “pseudoatoms.” Thus, when a proton is “added” to the O²⁻ ion in the nuclear sense, an isotope of fluorine is obtained (Figure 8.4).

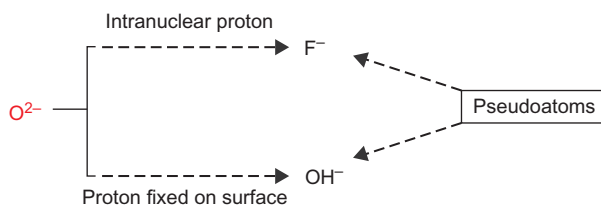


FIGURE 8.4 The notion of pseudoatoms.

TABLE 8.2 Hydride Displacement Law: In Each Vertical Column the Atom is Followed by its Pseudoatoms

Number of electrons					
6	7	8	9	10	11
C ⁴⁻	N ³⁻	–O–	–F	Ne	Na ⁺
	CH ³⁻	–NH–	–OH	FH	
		–CH ₂ –	–NH ₂	OH ₂	
			–CH ₃	NH ₃	OH ₃ ⁺
				CH ₄	NH ₄ ⁺

When the same proton is introduced at the peripheral electronic level, a “pseudo-F”—in other words, an OH[–]—is created. In this context, the H⁺ ion having penetrated the electronic shell of the oxygen is assumed to be masked by the greater atom and to exert only negligible effect toward the outside. The fluoride anion F[–] and the hydroxyl anion OH[–] show therefore some analogies. The generalization of the pseudoatom concept represents the so-called “hydride displacement law” proposed in a tabular form by Grimm [8] in each vertical column, the original atom is followed by its isosteric pseudoatoms (Table 8.2).

D. Erlenmeyer’s Expansion of the Isosterism Concept

Starting in 1932, Erlenmeyer [9] published a series of detailed studies about the isosterism concept, and particularly, about its first applications to biological problems. Erlenmeyer proposed his own definition of isosteres as “elements, molecules or ions in which the peripheral layers of electrons may be considered identical.”

Erlenmeyer also proposed three expansions of the isosterism concept:

1. To the whole group of elements present in a given column of the periodic table. Thus, silicon becomes isosteric to carbon, sulfur to oxygen, etc.
2. To the pseudoatoms, with the aim of including groups which at a first glance seem totally different, but which in practice possess rather similar properties. This is the case for the pseudohalogens (e.g., Cl ≅ CN ≅ SCN, etc.)
3. To the ring equivalents: the equivalence between –CH = CH– and –S– explaining the well-known analogy between benzene and thiophene (Table 8.3).

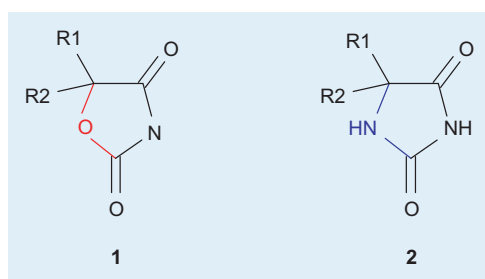
E. Isosterism Criteria: Present Conceptions

The main criterion for isosterism is that two isosteric molecules must present similar, if not identical, volumes and shapes. Ideally, isosteric compounds should be isomorphic and able to co-crystallize. Among the other physical properties that isosteric compounds usually share, one can cite boiling point, density, viscosity, and thermal conductivity. However, certain properties must be different: dipolar moments, polarity, polarization, size, and shape (e.g., in comparing F[–] and OH[–], the size and the shape of H cannot be totally neglected). After all, the external orbital may be hybridized differently.

In conclusion, it became evident to the physicists that the concept of isosterism, developed before quantum-mechanical theories, could not provide at the molecular level the same results as those that the periodic classification had provided for the elements, namely a correlation between electronic structure and physical and chemical properties. In the field of medicinal chemistry, the isosterism concept, taken in its broadest sense, has proven to

TABLE 8.3 The Sulphur Atom is Approximately Equivalent to an Ethylenic Group (Size, Mass, Capacity to provide an Aromatic Lone Pair)

$M_{(-CH=CH-)} = 26$ 		$M_{(S)} = 32$ 	
Compound	E°C	Isostere	E°C
Benzene	80°	Thiophene	84°
Methylbenzene	110°	2-Methyl-thiophene	113°
Chlorobenzene	132°	2-Chloro-thiophene	130°
Acetylbenzene	200°	2-Acetyl-thiophene	214°

**FIGURE 8.5** 5,5'-Disubstituted oxazolidine-diones **1** and hydantoins **2** show similar antiepileptic profiles.

be a research tool of the utmost importance. The main reason for this is that isosteres are often much more alike in their biological than in their physical and chemical properties. An illustrative example is found in the comparison of oxazolidine-diones (**1**) and hydantoins (**2**), which possess different chemical reactivities but present a similar antiepileptic profile (Figure 8.5).

F. The Bioisosterism Concept: Friedman's and Thornber's Definitions

Recognizing the usefulness of the isosterism concept in the design of biologically active molecules, Friedman [2] proposed to call bioisosteres compounds "which fit the broadest definition of isosteres and have the same type of biological activity." This definition received rapid acceptance and is now commonly used. Moreover, Friedman considers that isosteres that exhibit opposite properties (antagonists) have also to be considered as bioisosteres, since usually they interact with the same recognition site. This is the case for para-aminobenzoic acid and para-aminobenzene-sulfonamide [10,11] and also for glutamic acid and its phosphonic analogs [12,13].

The use of the word isosterism has been largely taken beyond its original meaning when employed in medicinal chemistry, and Thornber [14] proposes a loose and flexible definition of the term bioisostere: "Bioisosteres are groups or molecules which have chemical and physical similarities producing broadly similar biological effects."

III. CURRENTLY ENCOUNTERED ISOSTERIC AND BIOISOSTERIC MODIFICATIONS

In 1970, Burger [15] classified and subdivided bioisosteres into two broad categories: classic and nonclassic. Grimm's Hydride Displacement Law and Erlenmeyer's definition of isosteres outline a series of replacement that have been termed "classical bioisosteres." Classical bioisosteres [5,16] have been traditionally divided into several

TABLE 8.4 Classic Bioisostere Atoms and Groups

Monovalent	Divalent	Trivalent	Tetravalent
–OH, –NH ₂ , –CH ₃ , –OR	–CH ₂ –	=CH–	=C=
–F, –Cl, –Br, –I, –SH, –PH ₂	–O–	=N–	=Si=
–Si ₃ , –SR	–S–	=P–	=N ⁺ =
	–Se–	=As–	=P ⁺ =
	–Te–	=Sb–	=As ⁺ =
			=Sb ⁺ =

TABLE 8.5 Non-Classical Isosteres

–CO–	–COOH	–SO ₂ NH ₂	–H	–CONH–	–COOR	–CONH ₂
–CO ₂ –	–SO ₃ H	–PO(OH)NH ₂	–F	–NHCO–	–ROCO–	–CSNH ₂
–SO ₂ –	–tetrazole					
–SO ₂ NR–	–SO ₂ NHR		–OH		–catechol	
	–SO ₂ NH ₂		–CH ₂ OH			
–CON–	–3-hydroxyisoxazole				–benzimidazole	
–CH(CN)–	–2-hydroxychromones		–NHCONH ₂			C ₄ H ₄ S
R–S–R			–NH–CS–NH ₂			–C ₅ H ₄ N
(R–O–R)	=N–					–C ₆ H ₅
R–N(CN)–	C(CN)=R'		–NH–C(=CHNO ₂)–NH ₂			
			–NH–C(=CHCN)–NH ₂			
–halide						–C ₄ H ₄ NH
	–CF ₃					
	–CN					
	–N(CN) ₂					
	–C(CN) ₃					

distinct categories: (a) monovalent atoms or groups; (b) divalent atoms or groups; (c) trivalent atoms or groups; (d) tetravalent atoms, and (e) ring equivalents (Table 8.4).

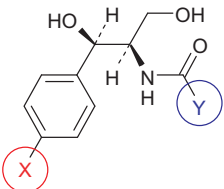
Some nonclassical isosteres are reported in Table 8.5 and from a brief glance it can be noticed that they do not obey the steric and electronic definition of classical isosteres. A second notable characteristic of nonclassical bioisosteres is that they do not have the same number of atoms as the substituent or moiety for which they are used as a replacement.

As the distinction between isosteres and bioisosteres is rather of academic interest, it is preferred in this chapter to treat both categories together. Consequently, for example, divalent series such as O=, HN=, and H₂C= can be discussed together with S=. However, the correct nomenclature will be used as much as possible, keeping in mind that “isosteric replacement” embraces both true isosteres and bioisosteres.

A. Replacement of Univalent Atoms or Groups

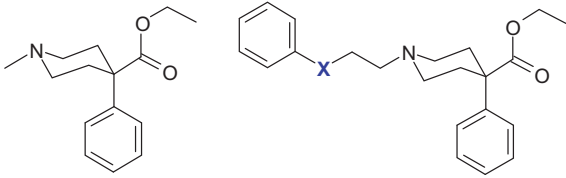
Halogens (particularly chlorine) can be replaced by other electron-attracting functions such as trifluoromethyl or cyano groups. In the antibiotic chloramphenicol, both the chlorine atoms of the dichloroacetic moiety and of the *para*-nitro-phenyl group yielded productive isosteric replacements (Table 8.6). Many other examples of univalent atoms or groups replacements are found in the chapter dealing with substituent effects (Chapter 20) and with quantitative structure–activity relationships (Chapter 23).

TABLE 8.6 Isosteric Replacements in the Amphenicol Family



Compound	X	Y
Chloramphenicol	–NO ₂	–CH–Cl ₂
Thiamphenicol	CH ₃ –SO ₂ –	–CH–Cl ₂
Cetophenicol	CH ₃ –CO–	–CH–Cl ₂
Azidamphenicol	–NO ₂	–CH ₂ –N ₃

TABLE 8.7 Meperidine Analogs [17]



X	Analgesic potency (Meperidine = 1)
O	12
NH	80
CH ₂	20
S	1,5

B. Interchange of Divalent Atoms and Groups

A first series of frequently interchanged divalent atoms or groups is represented by O, S, NH, and CH₂, and many interesting examples are found in the literature. In a study on meperidine analogs (Table 8.7), potent analgesic compounds were found for X = O, NH, and CH₂ [17]. Surprisingly, the sulfur analog showed only moderate activity. As an *in vivo* test was used to assess the activity, the weaker effect may be attributable to a faster metabolism (sulfoxide or sulfone formation?).

Similar changes can be applied to cyclic series, for example, to a series such as piperidine–morpholine–thiomorpholine–piperazine or in introducing oxygen or sulfur atoms into cyclic ketoprofen analogs [18].

Nice isosteric variations were observed in a series of thermolysin inhibitors [19]. For these isosteres the replacement of the phosphonamide (X = NH) function by a phosphonate (X = O) or a phosphinate (X = CH₂) function demonstrated clearly that the maximal activity was associated with the phosphonamide, which is able to establish a hydrogen bond with alanine 113 (Figure 8.6).

In order to have a more stable analog of the acetylcholinesterase inhibitor alkaloid physostigmine, Chen et al. [20] prepared some 8-carbaisosteres of physostigmine (Table 8.8). The authors envisioned that replacing the *N*-methyl group at N₈ of the physostigmine nucleus by a methylene group would increase its chemical and metabolic stability, thanks to the change of the less stable aminal group to a more stable amino group.

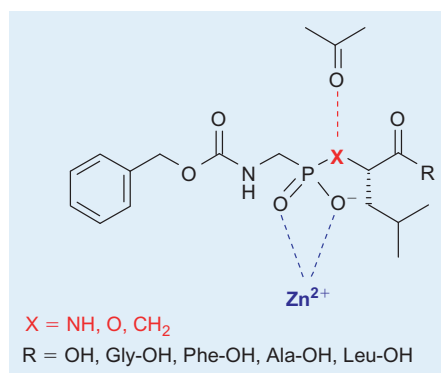
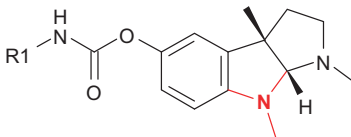
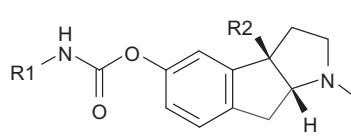


FIGURE 8.6 Isostery in thermolysin inhibitors [19].

TABLE 8.8 Physostigmines (a) and Carbaisosteres (b) [20]

				
	(a)		(b)	
Compound	R1	R2	IC ₅₀ (nM)	LD ₅₀ (mg/kg)
(-)-Physostigmine	CH ₃	CH ₃	128	0.88
(-)-Heptyl physostigmine	<i>n</i> -C ₇ H ₁₃	CH ₃	110	24
(±)-Carba-isostere 1	<i>n</i> -C ₇ H ₁₃	CH ₃	114	21
(-)-Carba-isostere 2a	<i>n</i> -C ₇ H ₁₃	C ₂ H ₅	36	6
(+)-Carba-isostere 2b	<i>n</i> -C ₇ H ₁₃	C ₂ H ₅	211	18

The carbaisosteres are as potent as or even more potent than the corresponding physostigmines. In addition, the (–)-enantiomers which possess the same absolute configuration at C_{3a} and C_{8a} as that of physostigmine, are generally six to twelve times more potent in inhibiting acetylcholinesterase than the corresponding (+)-enantiomers.

Other interesting bioisosteric replacements of oxygen atoms were devised during the search of non-hydrolyzable phosphotyrosyl (pTyr) mimetics. The phosphoryl ester oxygen of pTyr has been replaced either by a methylene (Pmp) [21] or by a difluoromethylene (F₂Pmp) [22].

C. Interchange of Trivalent Atoms and Groups

The substitution of –CH = by –N = in aromatic rings has been one of the most successful applications of classical isosterism (see Section III.D.). Interchange of trivalent atoms are found also in nonaromatic rings. For example the 4-dimethylamino-antipyrine and its carba-isostere are about as equally active as antipyretics [23] (Figure 8.7).

Similar interchanges are found in the proceeding from desipramine to nortriptyline and protriptyline (Figure 8.7), or among the antihistaminics when comparing tripelennamine with chlorpheniramine (Figure 8.7).

D. Ring Equivalents

The importance of chemical rings in drug discovery is never overstated. Looking at the best selling drugs in 2006 (reported in Table 8.9), eight out of ten molecules are small molecules and that all of them have a chemical ring in their structure. This trend is reflected also by the other marketed drugs and by the ensemble of bioactive molecules. In bioactive molecules, rings have a multiple role. Rings are responsible of the basic shape of the molecule, rendering it rigid or flexible, and are also responsible for a suitable spatial orientation of the pharmacophoric

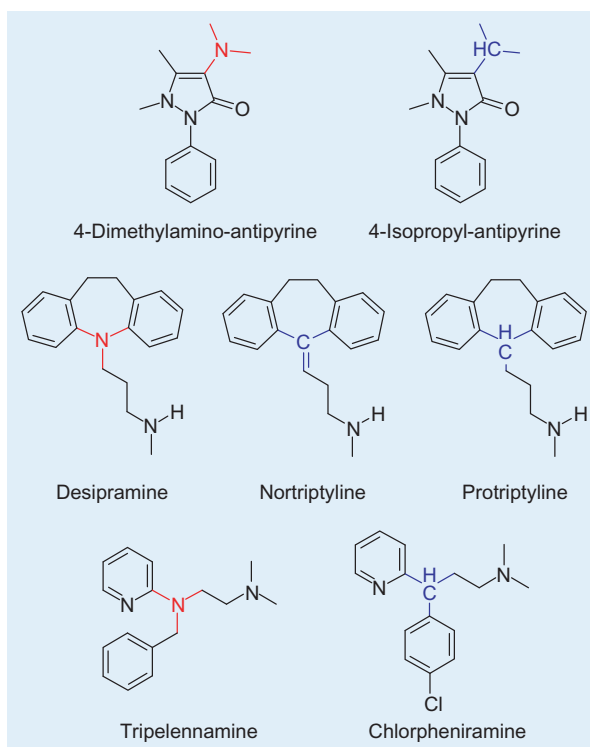


FIGURE 8.7 Interchange of trivalent atoms and groups.

groups. In other molecules, chemical rings are directly responsible of the biological activity, as they interact directly with the receptors either *via* heteroatom forming hydrogen bonding or *via* hydrophobic interactions.

The ADME (absorption, distribution, metabolism, excretion) profile as well as the toxicity of biologically active molecules can rely on the nature of a given ring, such as hydrophobicity, polarity, and electronic properties. Medicinal chemists use to change one ring with a bioisosteric one in order to improve the ADME-tox profile of a given molecule while keeping the desired activity and selectivity.

In the conception of “me too” drugs as well as in getting a stronger patent position, bioisosteric replacement of ring systems has played a master role in drug design. The substitution of $-\text{CH}=\text{CH}-$ by $-\text{N}=\text{N}-$ or $-\text{CH}=\text{CH}-$ by $-\text{S}-$ in aromatic rings has been one of the most successful applications of classical isosterism. Early examples are found in the sulfonamide antibacterial with the development of sulphapyridine, sulphapyrimidine, sulphathiazole, etc. (Figure 8.8).

Other examples are found in the neuroleptic or antidepressant tricyclics, in the benzodiazepine tranquilizers and antiepileptics, and in the development of semi-synthetic penicillins and cephalosporins with broader spectra of activity and greater stability toward β -lactamases. In some instances, an aromatic ring can be replaced by an ethynyl group. In some instances an aromatic ring can be replaced by an ethynyl group. Such a replacement was reported by Wallace et al. [24] in a series of inhibitors of Endothelin-converting Enzyme (ECE-1) deriving from the biphenyl compound CGS 26 303 and its analogs (Figure 8.8) [25].

In all these cases no essential activity difference is found between the original drug and its isostere. However, it can happen that the procedure fails. Binder et al. [26], for example, reported that thieno[2,3-*d*]isoxazole-3-methanesulfonamide, the thiophene analog of the anticonvulsant drug zonisamide (Figure 8.9) [27], was practically inactive against pentetrazole- or electroshock-induced convulsions in mice, even at high doses.

Due to the paramount role of ring systems in the drug discovery process, a lot of attention has been given to the equivalence between rings. Indeed the efforts to replace rings or other functional groups in an active molecule form a large part of medicinal chemistry practice devoted also to extend the space of bioactive molecules in the chemistry universe. A typical lead optimization program often involves a trial-and-error process of replacing chemical functionality, including rings, and in most cases this intellectual exercise is based on a chemist’s knowledge and recollection of his or her past experience. As a source of inspiration, the following paragraphs list some examples of bioisosteres of the most encountered rings in biologically active molecules.

II. LEAD COMPOUND DISCOVERY STRATEGIES

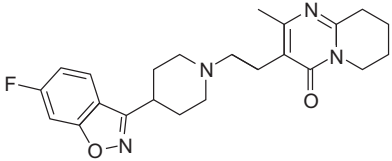
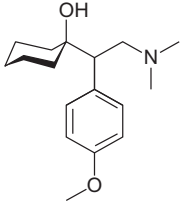
TABLE 8.9 Best Selling Drugs in 2006

Structure	Leading brands	Indication	Company	2006 sales (US\$BN)
	Lipitor (atorvastatin)	High cholesterol	Pfizer	13.6
	Nexium (esomeprazole)	Gastroesophageal reflux	Astra-Zeneca	6.7
	Seretide/Advair (fluticasone + salmeterol)	Asthma	GlaxoSmithKlin	6.3
	Plavix (clopidogrel)	Coronary artery disease	Sanofi-Aventis Brustol-Myers Squibb	5.8
	Norvasc (amlodipine)	Hypertension	Pfizer	5
165-Amino-acid-glycosylated protein hormone which contains 5 N-linked oligosaccharide chains	Aranesp (darbepoetin alfa)	Anemia	Amgen	5
	Zyprexa (olanzapine)	Schizophrenia	Eli Lilly	4.7

(Continued)

II. LEAD COMPOUND DISCOVERY STRATEGIES

TABLE 8.9 (Continued)

Structure	Leading brands	Indication	Company	2006 sales (US\$BN)
	Risperdal (risperidone)	Antipsychotic	Janssen Pharmaceutica	4.6
Recombinant human soluble tumor necrosis factor-alpha (TNF- α) receptor protein	Enbrel (etanercept)	Autoimmune diseases	Amgen-Wyeth	4.5
	Effexor (venlafaxine)	Depression	Wyeth	4

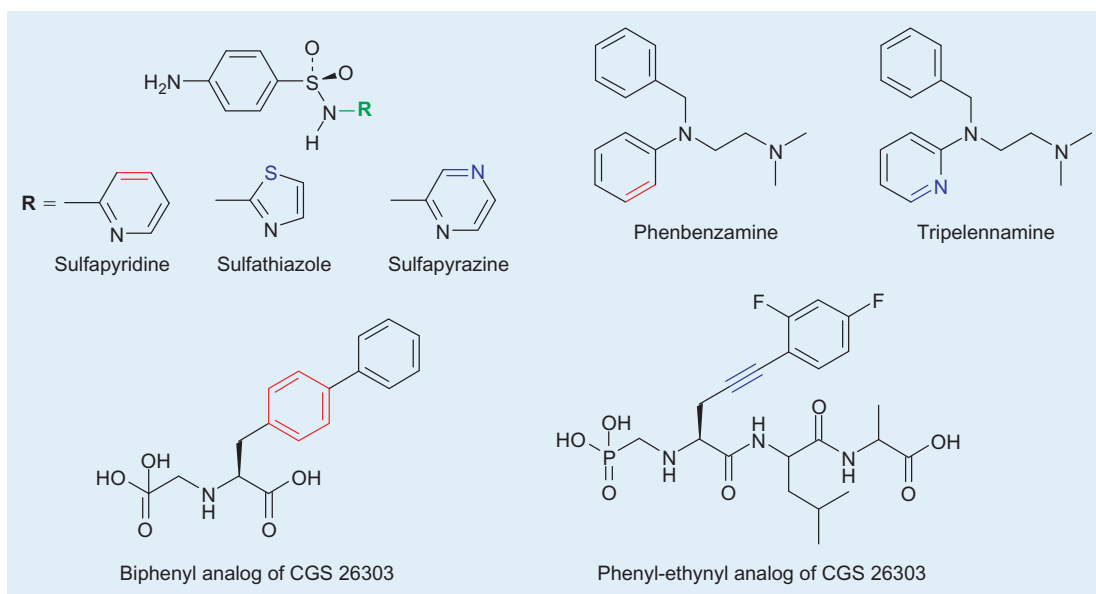


FIGURE 8.8 Classical ring equivalents.

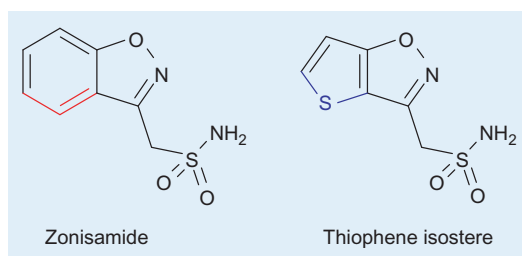


FIGURE 8.9 The thiophene isostere of zonisamide is practically inactive as an anticonvulsant.

II. LEAD COMPOUND DISCOVERY STRATEGIES

1. Bioisosteres of Pyridine

One of the most used cycles in medicinal chemistry is the pyridine ring. The bioisosteres of this heterocycle are well known as ligands for the central nicotinic cholinergic receptors. The pyridine ring of nicotine can be replaced by different other rings like methyl-isoxazole or methyl-isothiazole [28–30] (Figure 8.10a). A novel series of nicotinic agonists was described by Olesen et al. [30] In their paper, the bioisosteric replacement of the isoxazole ring in the (3-methyl-5-isoxazolyl)methylene-azacyclic compound (**3**, Figure 8.10b) with pyridine, pyrazine, oxadiazole, or an acyl group resulted in ligands with moderate to high affinity for the central nicotinic cholinergic receptors ($IC_{50} = 2.0$ to $IC_{50} > 1,000$ nM) (Figure 8.10b).

Two other publications on the bioisosteric replacement of pyridine show similar results. The first paper reports the study of bioisosteric potential of diazines in the field of combined antithrombic thromboxane A_2 synthetase inhibitors and receptor antagonists [31,32]. On the basis of the structure–activity relationships (SAR) observed in this study, it turned out that only the 2-pyrazinyl, 4-pyridazinyl, and 5-pyrimidinyl systems are appropriate bioisosteric moieties for the 3-pyridyl system in the dual active platelet antiaggregatory compound Ridogrel (Figure 8.11). Gohlke et al. [31] also observed the bioisosteric potential of diazines in the SAR of the compound

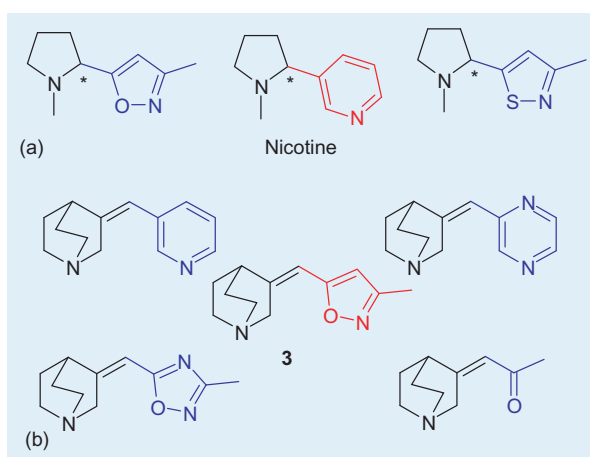


FIGURE 8.10 Ligands for central cholinergic receptors with different non-classical bioisosteres of the pyridine ring.

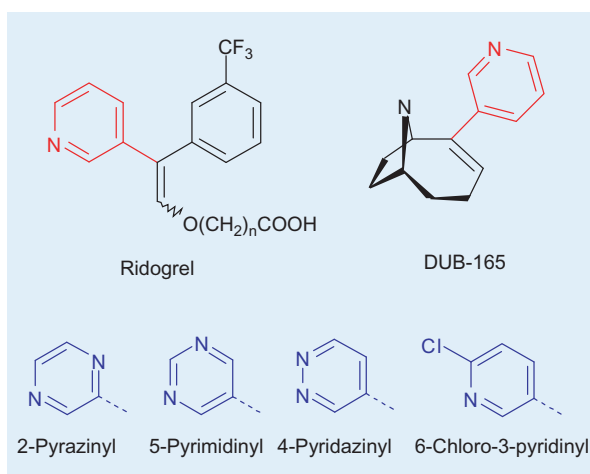


FIGURE 8.11 Bioisosteric moieties for the 3-pyridyl ring.

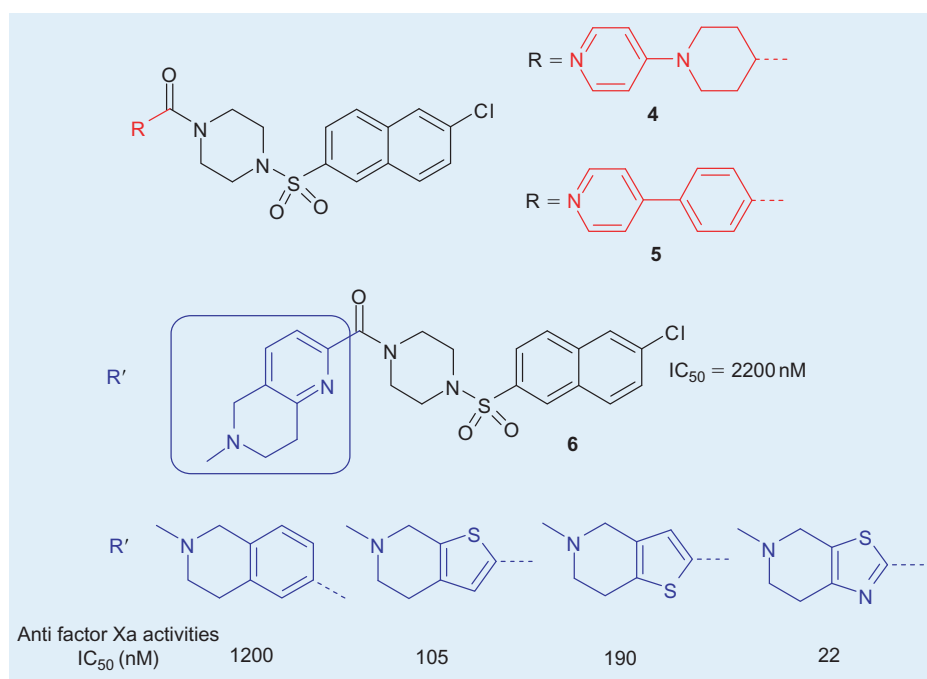


FIGURE 8.12 Bioisosteric replacements of the pyridine ring in a series of factor Xa inhibitors.

DUB-165. The replacement of the 3-pyridyl group of DUB-165 by a 4-pyridazinyl, 5-pyrimidinyl, or 2-pyrazinyl moiety resulted in ligands retaining affinity for nAChRs subtypes, thus demonstrating that the three isomeric diazines are appropriate bioisosteres of the 3-pyridyl moiety (Figure 8.11).

N-(6-Chloronaphthalen-2-yl)sulfonylpiperazine derivatives 4 and 5 (Figure 8.12) are potent factor Xa inhibitors. Haginoya et al. [33] proposed replacing the pyridine-phenyl or the pyridine-piperidine residue by a fused-bicyclic ring that contains an aliphatic amine and a pyridine to yield the compound 6, which has an interesting factor Xa inhibitor activity. The bioisosteric replacement of the pyridine moiety of the 6-methyl-5,6,7,8-tetrahydro-[1,6]naphthyridine by phenyl, thiophene, or thiazole analogs yielded analogs with similar or better antifactor Xa activity, but also to conserve a moderate bioavailability.

A nice example of pharmacophore equivalent of the 2,3-diaminopyridine with cyclopropylamino acid amide has been reported recently [34]. A series of 2,3-diaminopyridines represented by 7 acting as bradykinin (BK) B₁ antagonists was thoroughly investigated with the aim to replace the metabolically labile diaminopyridine ring with a more bioavailable moiety. An accurate design strategy led researchers at Merck to replace the 2,3-diamino pyridine with a series of alkylamino acid amides. Among this, series compound 8, though less active than 7 as BK B₁ antagonist, showed a better pharmacokinetic profile with an improved bioavailability, an increased half-life, and a decreased clearance compared to 7 (Figure 8.13).

To demonstrate the generality of this bioisosteric equivalence, the same group applied the same substitution to 9, a factor Xa inhibitor that yielded 10. Compound 10 reflects the same loss in potency that was observed for 8 towards 7, but reoptimization of the N-acyl group afforded quickly a significant gain of potency.

2. Imidazo[1,2-a]pyridine Bioisosteres

Abe et al. [35] reported that the imidazo [1,2-a]pyridine moiety of the basic framework of a class of the non-peptide bradykinin B₂ receptor antagonists (11; Figure 8.14) could be successfully replaced by several heterocyclic bioisosteres. Among those, the 1-methyl-2-methoxy-1H-benzimidazole, 2-methylquinoxaline and 2-methylquinoline derivatives showed potent B₂ binding affinities against both human and guinea pig B₂ receptors (Figure 8.14).

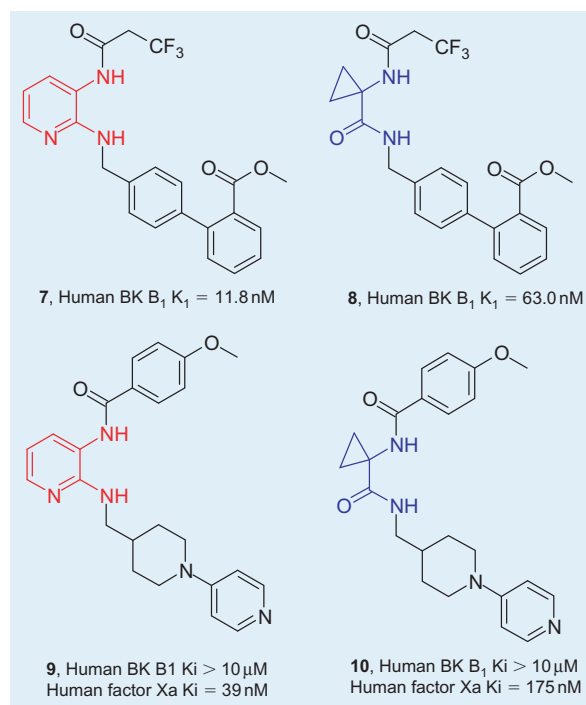


FIGURE 8.13 Cyclopropylamino acid amide as pharmacophoric replacement for 2,3-diaminopyridine. Application to the design of novel bradykinin B₁ receptor antagonist and factor Xa inhibitor.

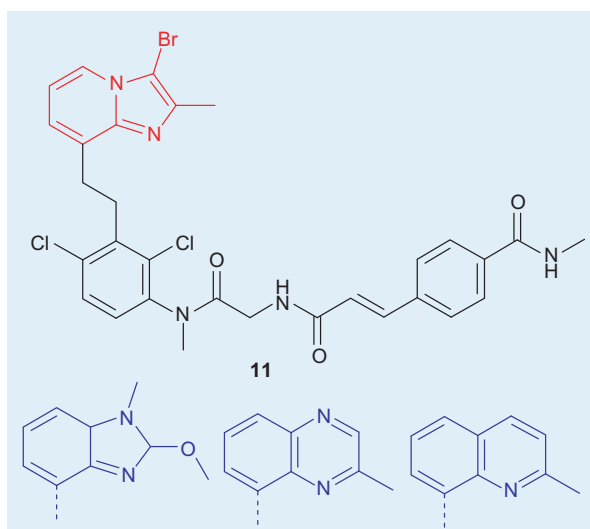


FIGURE 8.14 Novel class of orally active non-peptide bradykinin B₂ receptor antagonists showing valuable examples of imidazo[1,2-a]pyridine analogs.

3. Pyridazine Bioisosteres

Since the antidepressant minaprine has been launched, several pyridazine analogs were proposed and synthesized. Most of them showed different activities on the central nervous system. A series of these compounds (Figure 8.15) are acetylcholinesterase inhibitors with variable bioisosteres of the central pyridazine [36,37].

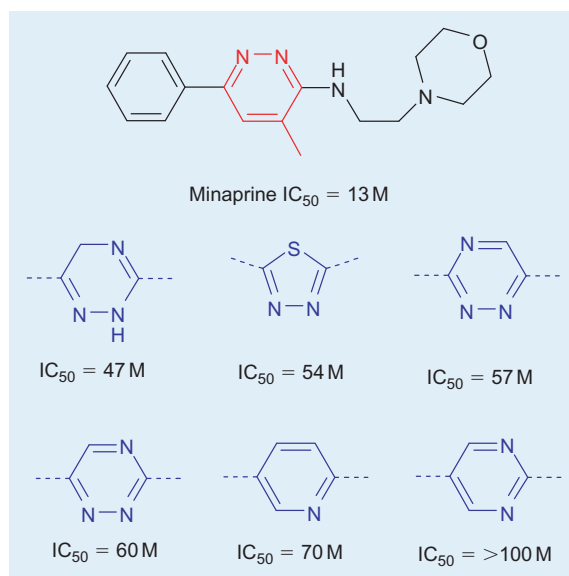


FIGURE 8.15 *In vitro* inhibition of acetylcholinesterase in rat striatum homogenates: pyridine, thiadiazole, and triazine replacement of the pyridazine ring.

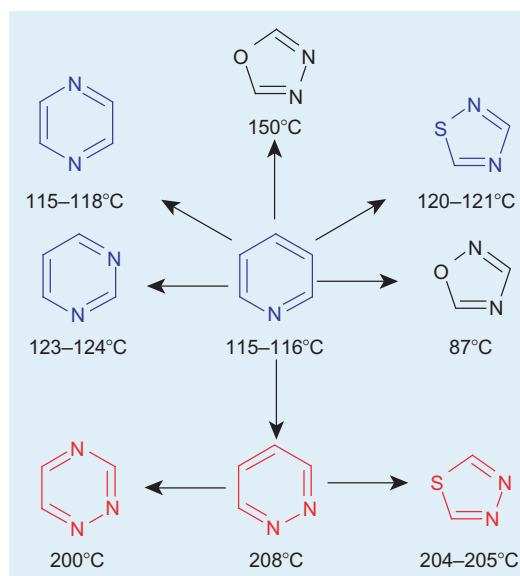


FIGURE 8.16 Structures and boiling points of pyridine and pyridazine isosteres.

The replacement by pyridine, 1,2,4-thiadiazole, and triazines yields compounds with weaker but still acceptable activity.

Examples from literature show that there are several nonclassical bioisosteres for the pyridine system or the pyridazine system. What is the basis for determining the most appropriate analog with which to start? An indication could be given by the comparison of the boiling points of these heterocycles, assuming that the more similar the boiling point is the most appropriate the bioisostere is (Figure 8.16) [38]. For example, while searching for a bioisostere of the pyridine ring, judging by the boiling points it appears that the best candidates are the pyrimidine, the pyrazine, and the 1,2,4-thiadiazole systems (Figure 8.16). The same could be extended to the pyridazine ring where this comparison permits the selection of the 1,2,4-triazine or the 1,3,4-thiadiazole rings (Figure 8.16).

II. LEAD COMPOUND DISCOVERY STRATEGIES

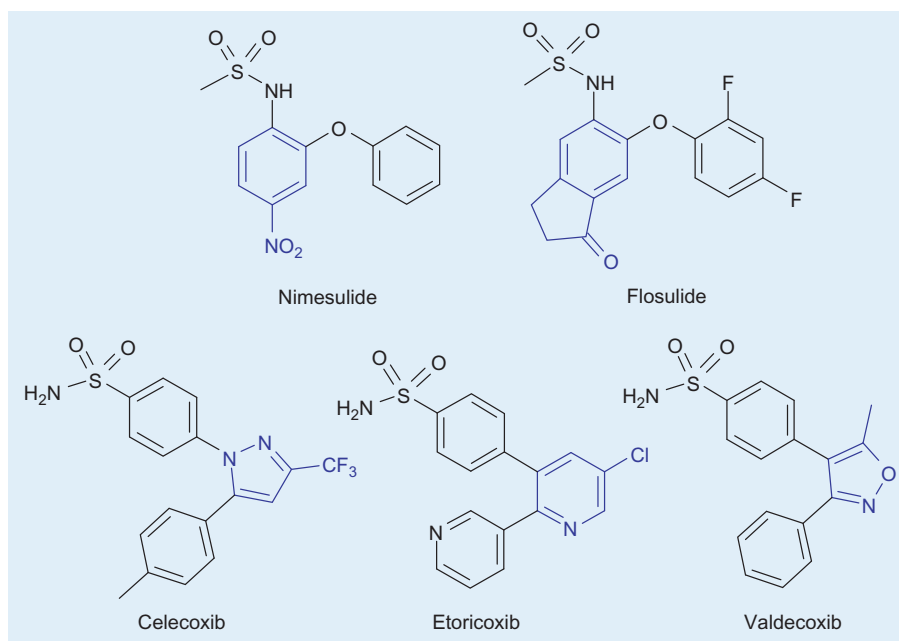


FIGURE 8.17 Examples of selective cyclooxygenase-2 inhibitors showing different ring equivalents.

In fact, those findings confirm the examples found in the literature and reported in the previous part of this chapter about isosteres or bioisosteres of the pyridine and pyridazine rings.

A possible interpretation of these results can be that in the heterocyclic series, the boiling point is correlated to the dipolar moment of the molecule and that for two heterocyclic rings having the same aromatic geometry, the similarity of the dipolar moments may represent the dominant feature.

4. Bioisosteres of Other Heterocycles

Chemical rings are fundamental for drug molecules as a typical drug molecule consists of a combination of chemical rings, chains, and functional groups. The high proportion of chemical rings in drug molecules speaks in favor of the large effort to synthesize and find new chemical ring equivalents, whether it is to design a new platform for a better patent position, replace a metabolically unstable moiety, look for a more favorable receptor interaction, or have a better pharmacodynamic profile.

There are many examples of ring equivalents, and so far it seems impossible to rationalize a method that could give a reliable indication of which heterocycle could be the best substitute of another one. The best approach is to look at the examples reported in the literature and learn from those.

Selective cyclooxygenase-2 inhibitors (COX-2 inhibitors) give a nice example of bioisosteres of heterocycles. The comparison of the most potent selective COX-2 inhibitors (Figure 8.17) suggests that isoxazoles, pyridines, and pyrazoles are good bioisosteres of each other as well as nitrophenol and indanones [39].

Another example of heterocycle bioisosteres is given by the oxazolidinone antibacterials. Since the discovery of linezolid as potent antibacterial against Gram positive organisms [40], many researchers have taken inspiration from the oxazolidinone scaffold in order to create new and potentially improved antibacterial agents. The structure of Linezolid (Figure 8.18) is divided in three parts: the A ring corresponding to the oxazolidinone moiety, the B ring corresponding to the Phenyl ring, and the C ring corresponding to the morpholine. Some research groups have worked around the modification of the B and C rings [41,42].

The work of Snyder et al, together with other examples found in the literature, shows that several levels of activity can be identified among the different isosteres. It has to be kept in mind that the replacement of a ring by its isosteres does not always lead to an iso-active or more active compound (Figure 8.18), but in general it is possible to obtain at least one or more equivalent systems [43].

Another particularly interesting example of ring bioisosterism is found in the development of the antiulcer H₂-receptor histamine antagonists in which the initial imidazole ring was changed to various other “equivalents”

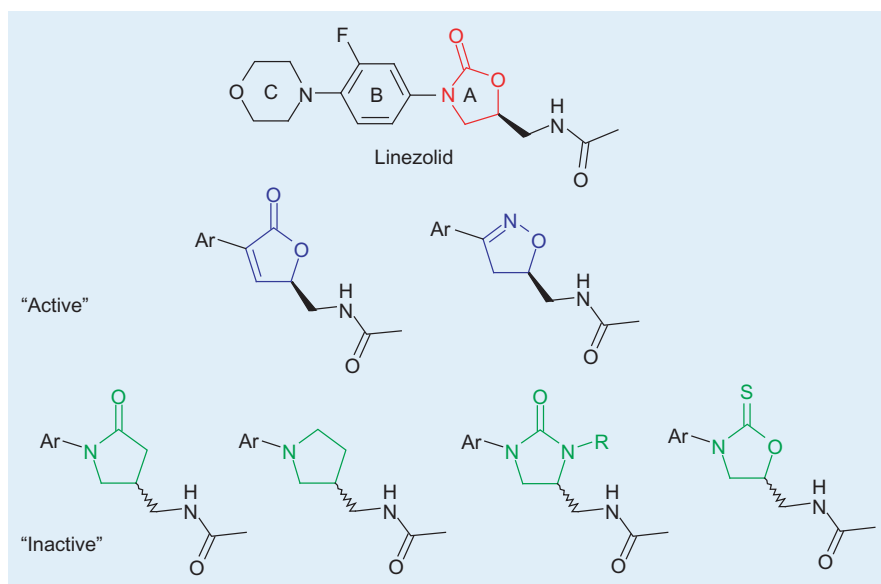


FIGURE 8.18 Oxazolidinone bioisosteres synthesized as antibacterial agents.

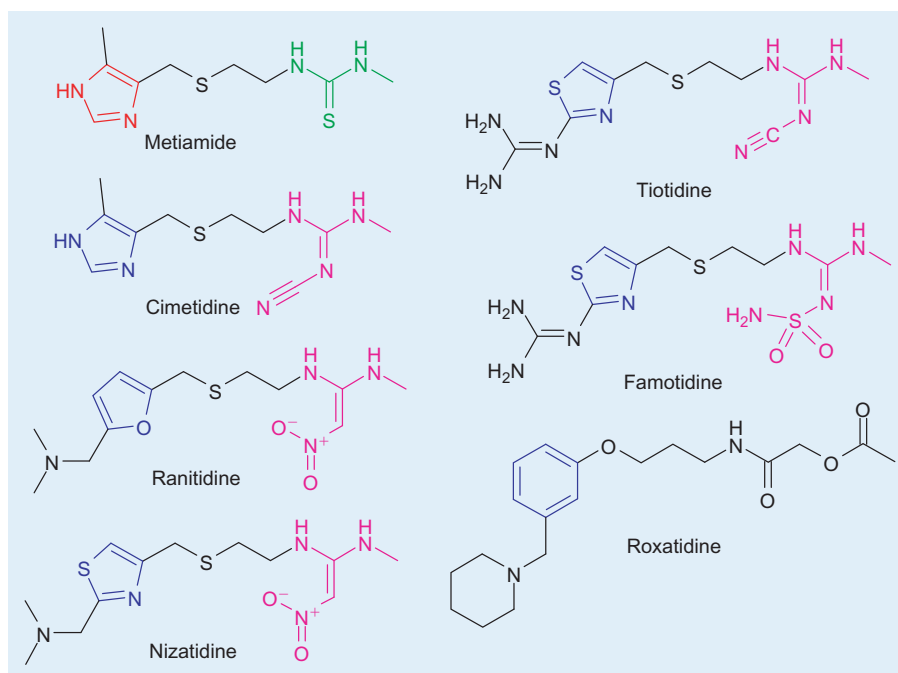


FIGURE 8.19 Antiulcer H_2 -receptor histamine antagonists: evolution of structures in the course of the time. Note the progressive use of a furan, a thiazole, and finally a phenyl ring in place of the original imidazole ring. On the same series it is noteworthy the bioisosterism of the urea moiety with different surrogates: thiourea, *N*-cyanoguanidine, *N*-nitro-ethene diamine, and *N*-aminosulfonyl guanidine.

such as a furan, a thiazole, and finally a phenyl ring (Figure 8.19). A detailed and interesting account of the discovery and the development of these compounds is found in Ganellin and Roberts' book [44].

Better bioisosteric design possibilities are provided by quantum-chemical calculations. Mallamo et al. [45] made use of electrostatic potential surface maps complementarity in defining sulfonyl heterocycles bioisosteric to the steroidal antiandrogenic drug zanerone (Figure 8.20). Striking differences in the electrostatic potential

II. LEAD COMPOUND DISCOVERY STRATEGIES

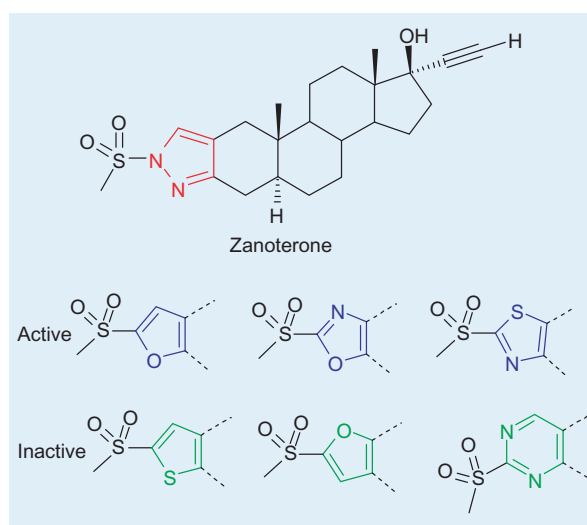


FIGURE 8.20 Zanterone isosteres [45].

surfaces accounted for the observed variability in the furan (active) and the thiophene (inactive) analogs of zanterone. Good androgen receptor affinity was then anticipated and effectively found for the oxazole and the thiazole analogs of zanterone.

The apparent failure of the isosterism concept for the inactive thiophene, inversed furan, and pyrimidine is thus interpretable on a rational basis. Table 8.10 lists some more “exotic” examples of bioisosteric replacements of cyclic systems.

E. Groups with Similar Polar Effects: Functional Equivalents

1. Carboxylic Acid Bioisosteres

Medicinal chemists have frequently faced the problem of developing surrogates for carboxylic acid groups. The availability of bioisosteric replacement for the carboxylic acid group has been critical to the development of novel medicinal agents, especially in the area of neurochemistry. Less polar and more hydrophobic groups were needed to replace the carboxylic group of GABA in order to cross the blood–brain barrier while keeping some of the physiochemical properties of the parent functional group like pK_a and partition coefficient.

Thus, the two GABAergic agonists, isoguvacine and THIP (Figure 8.21), have similar pharmacological properties to GABA itself. The key parameters in these compounds are the acidic ($pK_a < 4$) and the basic (protonated nitrogen) functions with a < 5.1 Å interchange distance.

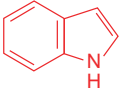
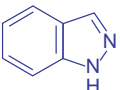
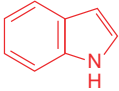
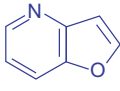
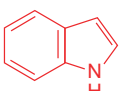
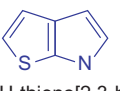
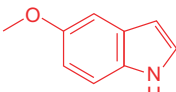
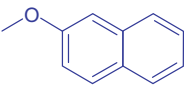
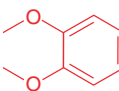
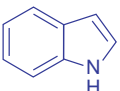
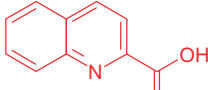
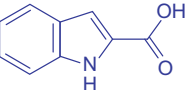
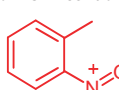
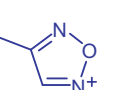
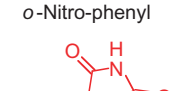
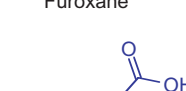
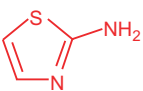
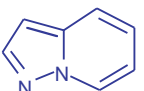
For the carboxylic function of active compounds, three different classes of bioisosteres can be distinguished: (a) the direct derivatives (Table 8.11); (b) the planar acidic heterocycles (Table 8.12); and (c) the nonplanar sulfur- or phosphorus-derived acidic functions (Table 8.5).

A. DIRECT DERIVATIVES

Direct derivatives of carboxylic acids are different functional groups that maintain an acidic proton and a hydrogen bond acceptor (the carbonyl) in order to have similar specific interactions with the receptor. Among the most common direct derivatives can be listed the hydroxamic acids ($R-CO-NH-OH$), the acylcyanamides ($R-CO-NH-CN$), and the acylsulfonamides ($R-CO-NH-SO_2-R'$) (Table 8.11).

Hydroxamic acids: The hydroxamic acids have a high chelating power, and they have found a wide application as inhibitors of enzymes having a metal ion like Zn^{2+} in the active site. Noteworthy examples are found in the inhibitors of matrix metalloproteinases (MMP) [65–67,86], tumor necrosis factor α converting enzyme (TNF α) [69], and histone deacetylase (HDAC) [59,60] where the hydroxamic acid derivatives have given potent and bioavailable compounds, despite the fact that this group is particularly prone to hydrolysis, reduction, and glucuronidation [68]. Another interesting example of exploitation of the hydroxamic acid is given by the anti-inflammatory hydroxamates bufexamac [87], ibuproxam [88], and oxametacin [89] (Figure 8.22), where the

TABLE 8.10 Other Ring Bioisosteres

Original ring	Bioisostere	Activity	Reference
 Indole	 Indazole	5-HT ₃ antagonists	Fludzinski et al. [46]
 Indole	 Furo[3,2-b] pyridine	5-HT _{1F} agonist	Mathes et al. [47]
 Indole	 6H-thieno[2,3-b] pyrrole	Hallucinogen serotonin agonist	Blair et al. [48]
 Indole	 Naphthalene	Agonist at melatonin receptors and antagonist at 5HT _{2c}	Yous et al. [49]; Depreux et al. [50]
 3,4-Dialkoxy-phenyl	 Indole	Phospho diesterase inhibitors GABA uptake inhibitors	Blaskó et al. [51]; Kardos et al. [52]
 Quinoline-2-carboxylate	 Indole-2-carboxylate	Glycine antagonists	Salituro et al. [53]
 o-Nitro-phenyl	 Furoxane	Calcium antagonist	Calvino et al. [54]
 Spiro-hydantoin	 Spiro hydroxyacetic acid unit	Aldose reductase inhibitor	Lipinski et al. [55]
 Thiazol-2-ylamine	 Pyrazolo[1,5-a] pyridine	Dopamine D ₃ receptor agonist	Löber et al. [56]

II. LEAD COMPOUND DISCOVERY STRATEGIES

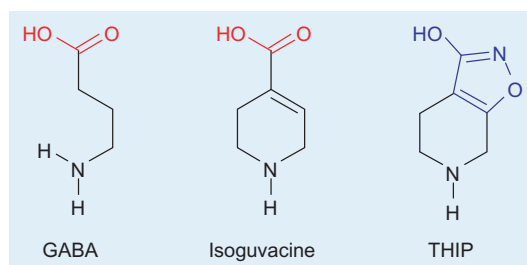


FIGURE 8.21 An example of bioisosterism, or non-classical isosterism: GABA, isoguvacine, and THIP are all agonists for the GABA-A receptor. The 3-hydroxy-isoxazole ring has a comparable acidity to that of a carboxylic acid function [57].

TABLE 8.11 Carboxylic Acid Isosteres: Direct Derivatives

	Hydroxamic acids	High chelating power	Almquist et al. [58]
		Histone deacetylase inhibitors	Massa et al. [59]; Lu et al. [60]
		Matrix metalloproteinases inhibitors	Remiszewski et al. [61]; Plumb et al. [62]; Kelly et al. [63]; Buggy et al. [64]
		Tumor necrosis factor α converting enzyme	Hanessian et al. [65]; Aranapakam et al. [66,67]; Noe et al. [68]; Duan et al. [69]
	Acyl-cyanamides	Mainly academic interest	von Kohler et al. [70]; Kwon et al. [71]
	Acyl-sulfonamides	Glycine, GABA, and β -alanine analogs	Drummond and Johnson [72]
		Antiatherosclerotics pK_a # 4,5	Albright et al [73]
		β_3 Adrenergic receptor agonist hepatitis C virus	Uehling et al. [74]; Johansson et al [75]

hydroxamates can act either as a prodrug-like ibuprofen being metabolized to ibuprofen ($\text{CONHOH} \rightarrow \text{COOH}$) in humans [90] or as a true bioisostere like oxametacin being metabolically stable in humans [91,92].

Acylsulfonamides: Acylsulfonamides are also used in drug discovery and have been employed in several therapeutic areas such as β_3 adrenergic receptor agonist [74], chemokine receptor 1 (CXCR1) inhibitors [93], hepatitis C virus (HCV) inhibitors [75], angiotensin receptor agonists [94], antitumoral compounds with antiproliferative properties [95,96], and prostaglandin EP4 receptor antagonists [97]. Acylcyanamides are mainly of academic interest.

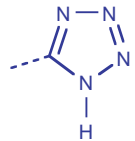
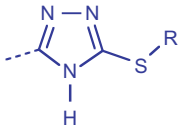
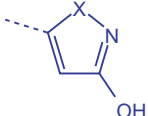
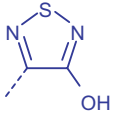
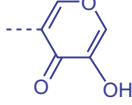
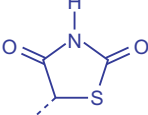
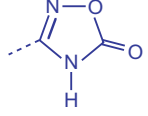
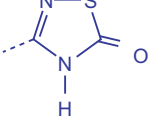
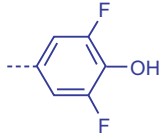
B. PLANAR ACIDIC HETEROCYCLES AND ARYL DERIVATIVES

Table 8.12 reports the planar acidic heterocycles and aryl derivatives that are commonly used as carboxylic acid bioisosteres. The most used is the tetrazole ring, which has found wide application in different therapeutic fields spanning type 2 diabetes [98,99], hepatitis C virus (HCV) [75], malaria [100], Alzheimer's disease (AD) [101], anxiety treatment [102], and pain management [103]. The tetrazole has been employed to fix different issues, for example, to improve bioavailability [100], to enhance the blood–brain barrier penetration [102,103], to increase potency [99,104], to get a better chemical stability [101], to bring some selectivity (the GABA tetrazole analog inhibits GABA-transaminase, but not succinic semialdehyde dehydrogenase) [105], or to be used as a pro-drug [106]. However, in some instances, tetrazole analogs are poorly active [107].

Hydroxy-isoxazoles and other cognate heterocyclic phenols encompassing an acidity range from 3.0 to 7.1 were incorporated in GABA agonists, antagonists, and uptake inhibitors [108,109]. The experience gained with 3-hydroxy-isoxazoles in the GABA field was also transferable to glutamate receptor ligands and led to selective antagonists for glutamic acid receptor subtypes [108].

Thiazolidinediones are another class of heterocyclic carboxylic acid surrogates commonly used for peroxisome proliferator-activated receptors (PPAR) agonists [81,82], as potent antihyperglycemic and lipid activity modulators. Other interesting but less studied heterocyclic surrogates are: 3,5-dioxo-1,2,4-oxadiazolidine [110],

TABLE 8.12 Carboxylic Acid Isosteres: Planar Acidic Heterocycles and Aryl Derivatives

	Tetrazoles	Very popular, great number of publications. $pK_a = 6.6$ to 7.2	Bovy et al. [76]; Marshall et al [77]
	Mercaptoazoles + Sulfinylazoles + Sulfonylazoles	Phosphonate isosteres pK_a mercapto: 8.2 – 11.5 pK_a sulfinyl: 5.2 – 9.8 pK_a sulfonyl: 4.8 – 8.7	Chen et al. [20]
	Isoxazoles Isothiazoles	GABA and glutamic acid analogs	Krogsgaard-Larsen et al. [57] Krogsgaard-Larsen [78]
X = O or S			
	Hydroxy-thiadiazole	Isoxazole isostere pK_a # 5	Lunn et al. [79]
	Hydroxy-chromones	Kojic acid derivatives (as GABA agonists)	Atkinson et al. [80]
	Thiazolidinediones	Dual PPAR α/γ agonists	Hulin et al. [81] Henke [82]
	1,2,4-Oxadiazole-5(4H)-ones	Antimycobacterial	Gezginci et al. [83]
	1,2,4-Thiadiazole-5(4H)-ones	Antimycobacterial	Gezginci et al. [83]
	3,5-Difluoro-4-hydroxyphenyl	Aldose reductase inhibitor GABA analog	Nicolaou et al. [84] Qiu et al. [85]

3-hydroxy-1,2,5-thiadiazoles [79], 1,2,4-oxadiazole-5(4H)-ones [83], 1,2,4-thiadiazole-5(4H)-ones [83], 3,5-difluoro-4-hydroxyphenyl [84,85], and 3-hydroxy- γ -pyrones [80,111].

C. NONPLANAR SULFUR- OR PHOSPHOROUS-DERIVED ACIDIC FUNCTIONS

The most extensive use of phosphonates (Table 8.13) occurred in the design of amino acid neurotransmitter antagonists such as glutamate [114] and GABA-B antagonists [112].

Among a set of cholecystokinin (CCK) antagonists derived from the nonpeptide CCK-B selective antagonist CI-988, Drysdale et al. [115] prepared a series of carboxylate surrogates spanning a pK_a range from <1 (sulfonic

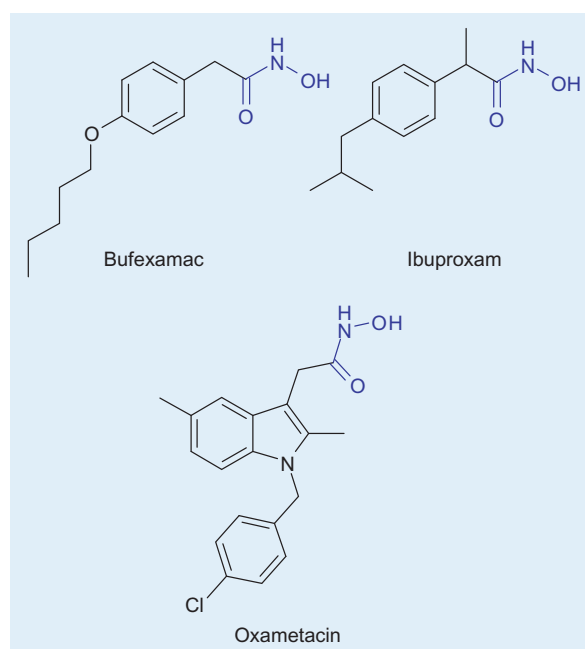


FIGURE 8.22 Hydroxamate isosteres of antiinflammatory drugs.

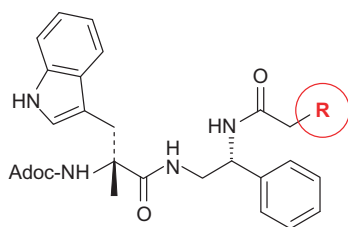
TABLE 8.13 Carboxylic Acid Isosteres: Non Planar Sulfur- or Phosphorus-Derived Acidic Functions

	X = H	Phosphinates	Many examples in glutamate and in GABA _B antagonist series	Froestl et al. [112]
	X = OH	Phosphonates		
	X = NH ₂	Phosphoramidates		
	X = CH(OR) ₂	Phosphonamides		
		Sulfonates	Sulphonic analogs of GABA and glutamic acid	Rosowski et al. [113]
		Sulfonamides	Weak acids, used rather as equivalents of phenolic hydroxyls in the design of catecholamine analogs	von Kohler et al. [70]

acid) to >9.5 (thio-1,2,4-triazole). The affinity and the selectivity of the compounds were rationalized by considering pK_a values, charge distribution, and geometry of the respective acid mimics (Table 8.14).

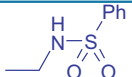
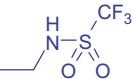
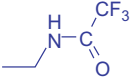
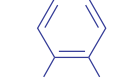
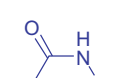
In order to choose the best carboxylic acid bioisoster, pK_a and $\log P$ are important parameters to examine before proceeding with one surrogate or another. An interesting study comparing pK_a and $\log P$ values of some aryl phosphonic acids, aryl tetrazoles, and aryl sulfonamides has been published by Franz [116]. The values of pK_a and $\log P$ are very important parameters in drug design. Several published examples show that the interchange of carboxylic acid for tetrazole and sulfonamide often results in useful drugs. This study indicates that the $\log P$ will be lowered by about 1 log unit by substituting a phosphonic acid by a carboxylic acid group. There is an increase in acidity that may be a limiting factor in the absence of active transport. Replacing a carboxylic acid group with a phosphonic acid group gives a much more acidic compound; replacing it with a sulfonamide group results in a much less acidic compound; and with a tetrazole replacement, acidity is essentially unchanged (Figure 8.23). If one wants to lower the $\log P$, and if increased acidity of the compound is not a limiting factor, substitution of a phosphonic acid group for a carboxylic acid would be a viable approach (Figure 8.24).

II. LEAD COMPOUND DISCOVERY STRATEGIES

TABLE 8.14 Exploration of the Carboxyl Isosterism Possibilities in a Series of CCK Antagonists

R	IC ₅₀ (nM) CCK-B	IC ₅₀ (nM) CCK-A	A/B ratio	pK _a
R-CH ₂ -COOH	1.7	4500	2500	5.6
<i>CHARGE DISTRIBUTED MONOANIONIC ACID MIMICS</i>				
	6.0	970	160	5.4
	2.6	1700	650	6.5
	2.4	620	260	4.3
	2.5	680	270	>9.5
	16	850	53	>9.5
	4.3	660	150	7.7
	1.7	940	550	7.0
	6.3	1300	200	5.2
	18	600	33	>8.2
	14	1300	93	>9.5

II. LEAD COMPOUND DISCOVERY STRATEGIES

R	IC ₅₀ (nM) CCK-B	IC ₅₀ (nM) CCK-A	A/B ratio	pK _a
<i>POINT CHARGE MONOANIONIC ACID MIMICS</i>				
	70	300	4.3	>9.5
	77	680	9	7.9
	110	790	7	>9.5
	80	510	6.4	>9.5
	21	1500	71	>9.5
<i>TETRAHEDRAL ACID MIMICS</i>				
P(O)(OH) ₂	27	5200	190	3.4; 7.7
CH ₂ -P(O)(OH) ₂	23	2700	120	3.4; 7.8
P(O)(OH)(OEt)	12	480	40	6.5
P(O)(OH)Me	12	1700	140	3.8
CH ₂ -P(O)(OH)Me	23	4400	190	3.7
CH ₂ -SO ₃ Na	1.3	1010	780	—

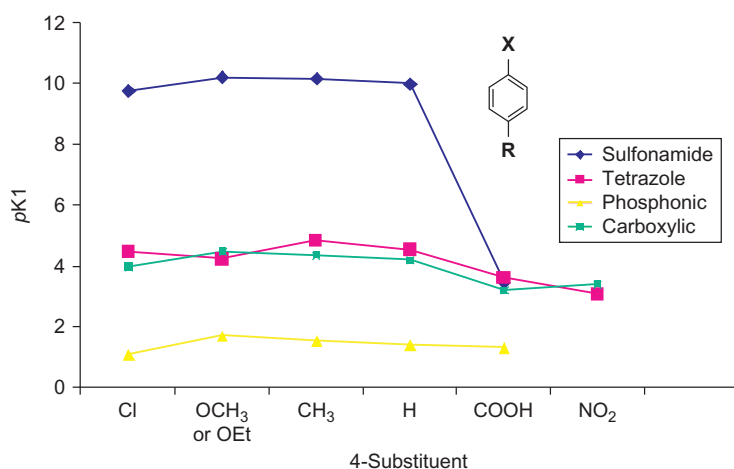


FIGURE 8.23 pK₁ values for aromatic compounds. Redrawn after Franz [116].

Diamino-cyclobutene-diones as α -amino carboxylic acid surrogates: Diamino-cyclobutene-dione was proposed by Kinney et al. [117] as an original surrogate of the α -amino carboxylic acid function (Figure 8.25).

Carboxylic functions as phosphonates surrogates: pTyr mimetics serve as important components of many competitive protein-tyrosine kinases inhibitors. To date, the most potent of these inhibitors have relied on phosphonate-based structures to replace the 4-phosphoryl group of the parent pTyr residue (Figure 8.26). Interestingly, it was found that carboxy-based pTyr analogs can be utilized to introduce the anionic oxygen functionality of the parent phosphate. Particularly, when *p*-(2-malonyl)phenylalanine (Pmf) was incorporated as pTyr replacement in the high-affinity Grb2 SH2 domain binding sequence, potencies approaching one of phosphonate mimetics were obtained [118].

II. LEAD COMPOUND DISCOVERY STRATEGIES

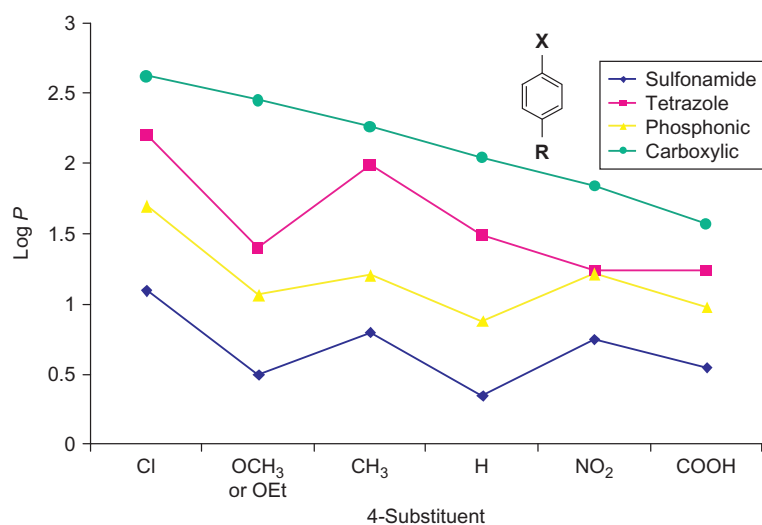


FIGURE 8.24 Log *P* values for aromatic compounds. Redrawn after Franz [116].

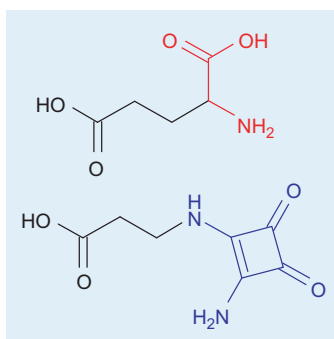


FIGURE 8.25 3,4-Diamino-3-cyclobutene-1,2-dione as surrogate of the α -amino carboxylic acid function.

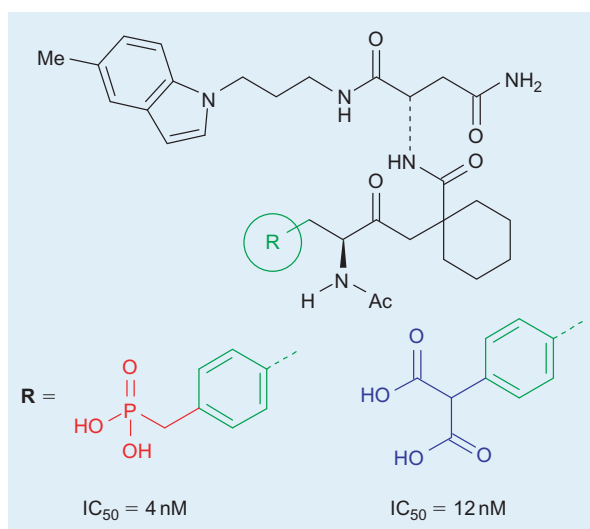


FIGURE 8.26 Malonates as surrogates of phosphonates.

II. LEAD COMPOUND DISCOVERY STRATEGIES

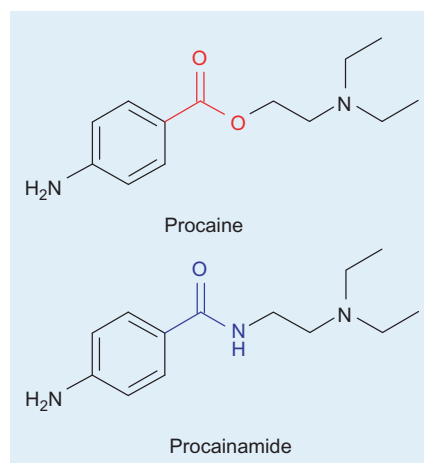


FIGURE 8.27 Amide as an ester surrogate of the local anesthetic procaine.

The above example is an elegant illustration of the possibility to mimic the pyramidal structure of phosphates or phosphonates by means of two planar carboxylic groups, the three-dimensionality originating from the malonic methylenic carbon atom.

2. Carboxylic Esters Bioisosteres

Carboxylic esters are nice tools for SAR studies investigation but they are not a valuable group to be left in drug molecules because they lack the proper of *in vivo* stability. As a matter of fact, they are hydrolyzed quite quickly by the esterases that are quite ubiquitous and are present in the blood, the liver, the kidneys, and other organs. To overcome this problem, the carboxylic ester in the most promising compounds has been replaced by bioisosteric groups leading to molecules with improved pharmacokinetic profile.

One of the first examples of ester bioisoster is the amide bond as it is illustrated by the local anesthetic procaine, where the readily hydrolyzable ester group was replaced with an amide to give procainamide as a more stable analog (Figure 8.27).

Similarly, the lactone ring of the muscarinic agonist pilocarpine was changed into various still active isosteres, such as the corresponding thiolactone, lactam, lactol, and thiolactol [119]. A series of aspirin isosteres has been prepared by replacing the carboxylic ether oxygen successively by a nitrogen, sulfur, or carbon isosteric equivalent [120]. None of the isosteric compounds showed any activity. This result is readily understood since the particular role of aspirin as an acylating agent of the enzyme cyclooxygenase has been demonstrated [121].

Pilocarpine is widely used as a topical miotic for controlling the elevated intraocular pressure associated with glaucoma. Besides its low lipophilicity, which stimulated the search for prodrugs [122], pilocarpine has a short duration of action, its lactonic ring being rapidly opened to yield pilocarpic acid. In pilocarpine, by substituting the lactonic ester function with its carbamate equivalent, a much more stable analog—which is as effective as pilocarpine—was obtained [123].

In addition to these classical changes, more exotic groups and radicals have been proposed and tested as valuable carboxylic ester surrogates, like five-membered heterocycles and alkyl oximes. Studies about the design and the synthesis of more metabolically stable muscarinic ligands have shown that it is possible to replace the methyl ester of arecoline **12** and of the quinuclidine **13** with a series of five-membered rings like oxadiazoles, thiadiazoles, triazoles, and tetrazoles to give potent muscarinic agonist (Figure 8.28) [124–130].

Worth noting is the successful replacement of the methyl ester with oxime ethers and *N*-methoxy imidoyl nitrile in the design of muscarinic agonists (Figure 8.28) [131,132].

Several 1,2,4-oxadiazoles and the other five-membered heterocycles are employed for other therapeutic indications, like monoamine transporter and opioid receptors [133,134], 5-HT agonists [135], bradykinin B₁ receptor antagonists [136], and vascular endothelial growth factor receptor (VEGFR-2) inhibitors [137].

Recently, a nice example of the use of the methyl ester bioisosteres in the design of a series of metabolically stable HIV inhibitors has been published (Figure 8.29) [138,139]. Starting from an alkenyldiarylmethane containing three methyl esters, the systematic substitution of an ester at the time with the appropriate biomimetic group

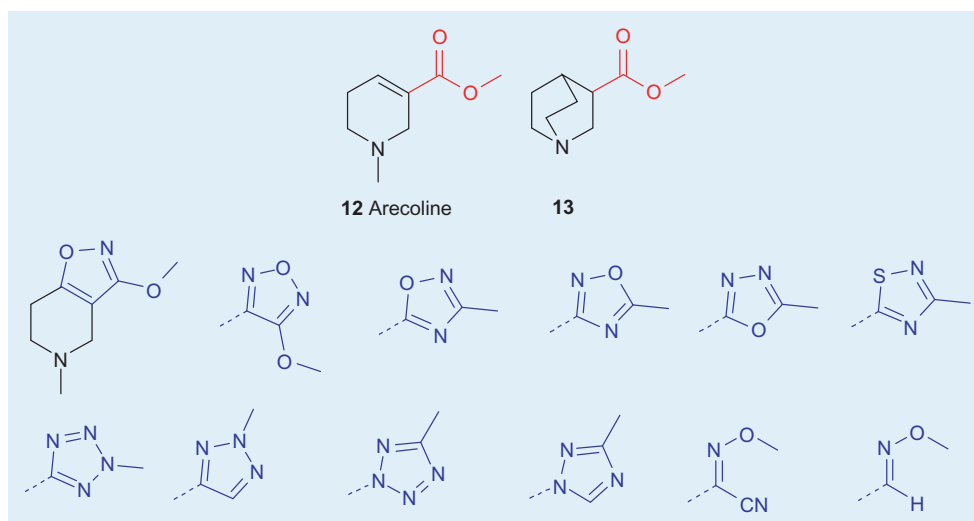


FIGURE 8.28 Muscarinic ligands derived from the ester bioisoster replacement of arecoline 12 and quinuclidine methyl ester 13.

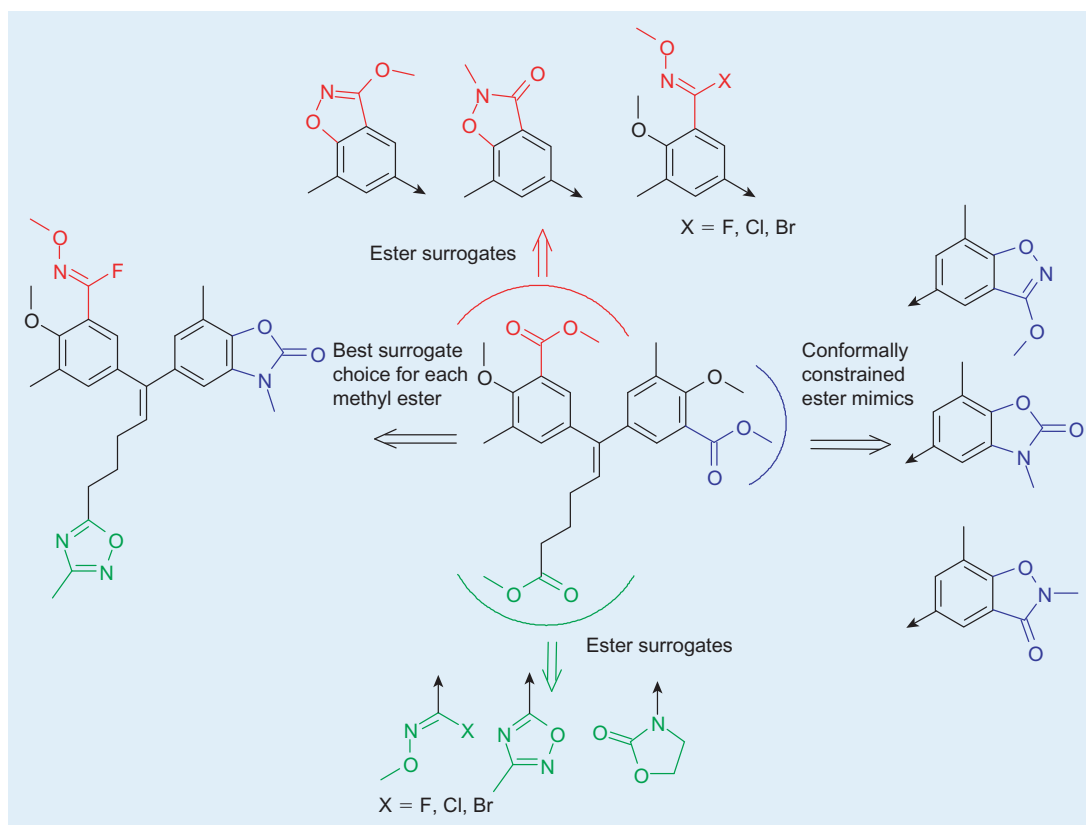


FIGURE 8.29 Design of new metabolically stable HIV inhibitor.

II. LEAD COMPOUND DISCOVERY STRATEGIES

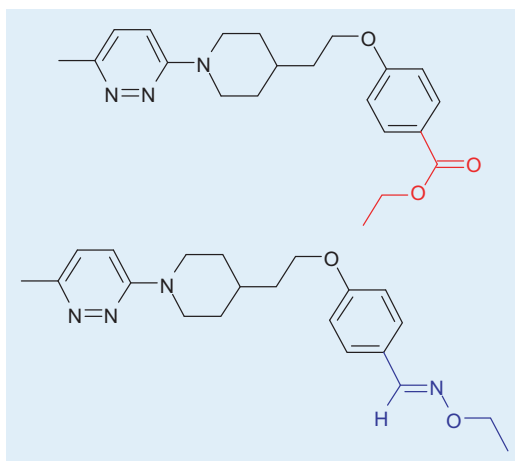


FIGURE 8.30 Ethyl oxime ether as ester replacement gave an improved bioavailability.

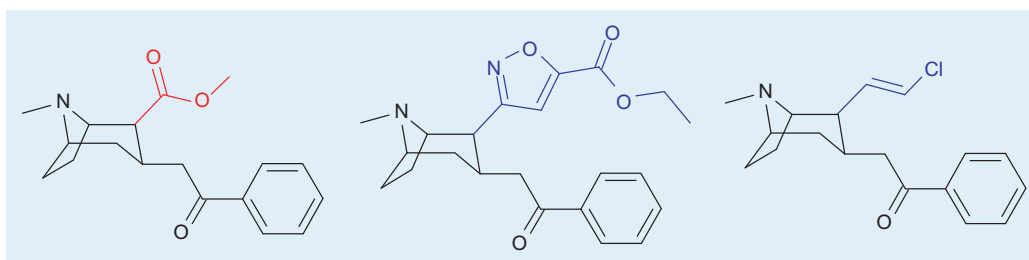


FIGURE 8.31 Replacement in (–)-cocaine of the carbomethoxy group by a carbethoxyisoxazole and a chlorovinyl moiety.

led to a molecule with an enhanced metabolic stability in rat plasma ($t_{1/2}$ = 61 hour) along with the ability to inhibit HIV-1 reverse transcriptase (Figure 8.29).

A confirmation of the alkyl oxime ether as metabolically stable isoster of an ester has been reported by Watson et al. in the synthesis of a capsid binder active against Human Rhinovirus [140] (Figure 8.30).

The change in (–)-cocaine of the carbomethoxy substituent into carbethoxyisoxazole doubles the potency in [^{3} H] mazindol binding and [^{3} H] dopamine (DA) uptake (Figure 8.31). Astonishingly, the replacement of the carbomethoxy group by a chlorovinyl moiety produces a comparable gain in potency, thus arguing against the involvement of the carbomethoxy group in H-bonding [141].

Another rather unusual example of ester isosterism is the replacement of the ether oxygen by a fluoronitrogen (Figure 8.32a) as mentioned by Lipinski [142]. Other uncommon examples are found in the replacement of the ester function of acetylcholine by exo–endo amidinic functions of 3-aminopyridazines in muscarinic agonists (Figure 8.32b) [143] and of the carbomethoxy group of α -yohimbine (rauwolscine) by a *N*-methylsulfonamide function (Figure 8.32c) [144].

3. Carboxamide Bioisosteres

Biologically active molecules containing amide bonds usually suffer from pharmacokinetic liability. In order to increase their stability, bioisosteric transformation of the carboxamide have been performed and yielded a lot of successful examples, especially in the area of peptidomimetic. The isosteric replacements for peptidic bonds have been summarized by Spatola [145] and by Fauchère [146]. The most used and well-established modifications are: *N*-methylation, configuration change (*D*-configuration at $C\alpha$), formation of a retroamide or an α -azapeptide, use of aminoisobutyric or dehydroamino acids, replacement of the amidic bond by an ester [depsipeptide], ketomethylene, hydroxyethylene or thioamide functional group, carba replacement of the amidic carbonyl, and use of an olefinic double bond (Figure 8.33).

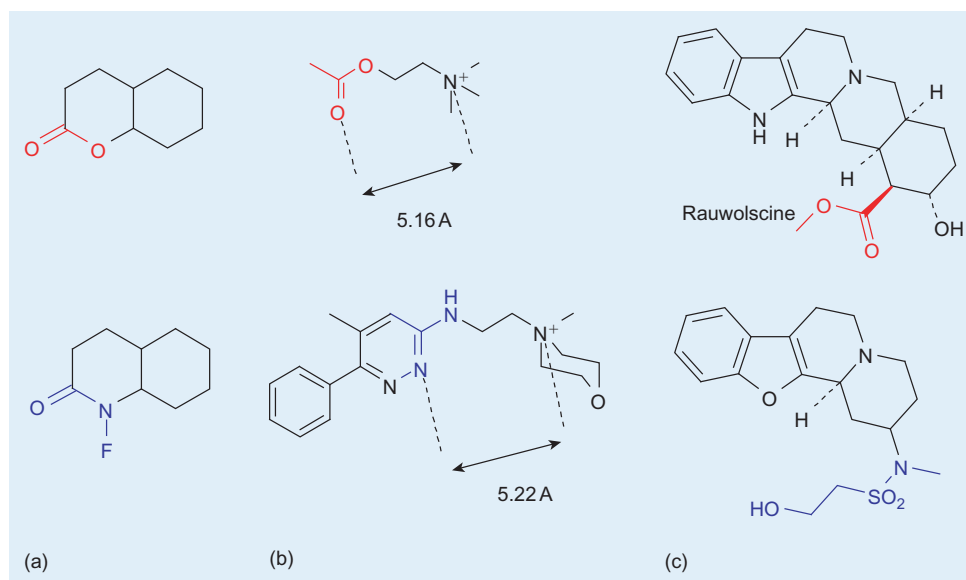


FIGURE 8.32 (a) Replacement of ester ether oxygen by a fluoro-nitrogen; (b) Exo-endo amidine in place of a carboxylic ester functionality; (c) *N*-methylsulfonamide analog of α -yohimbine (rauwolscine).

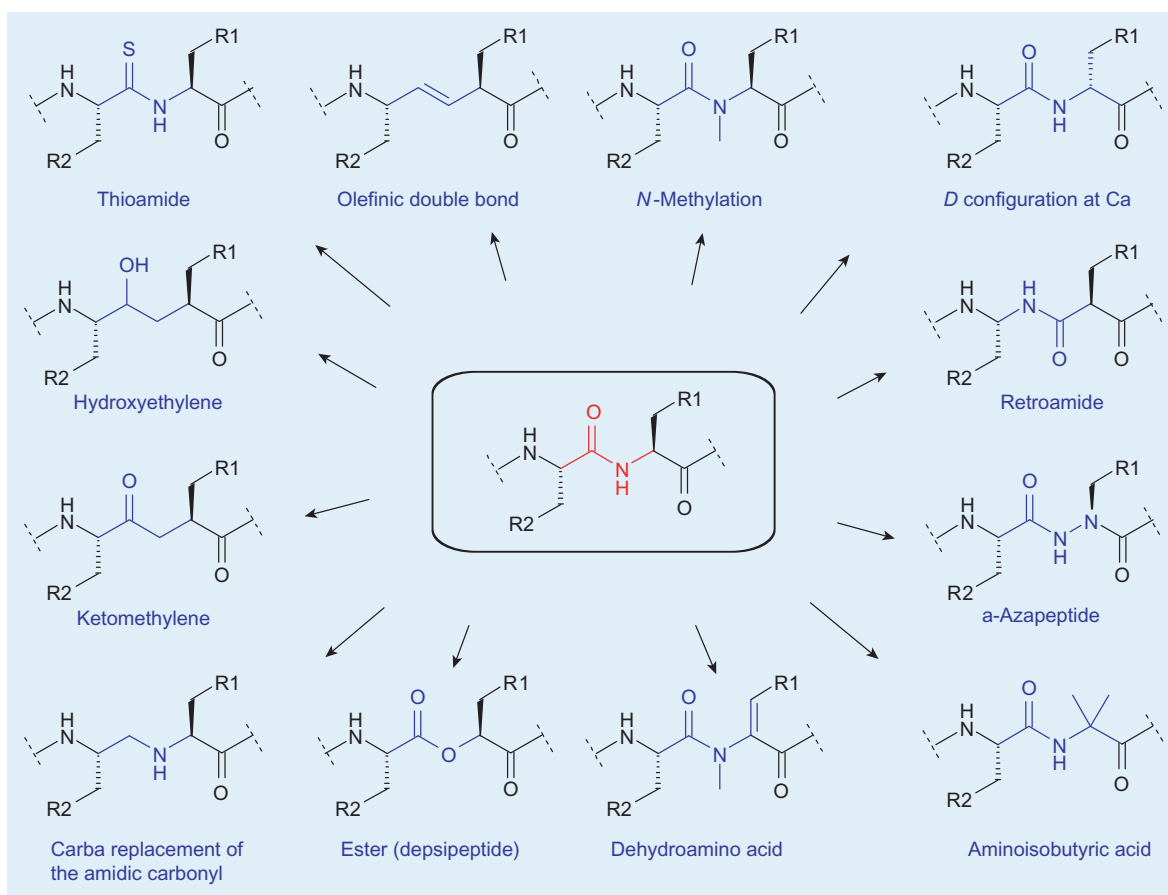


FIGURE 8.33 Well-established isosteric replacements for peptidic bonds.

II. LEAD COMPOUND DISCOVERY STRATEGIES

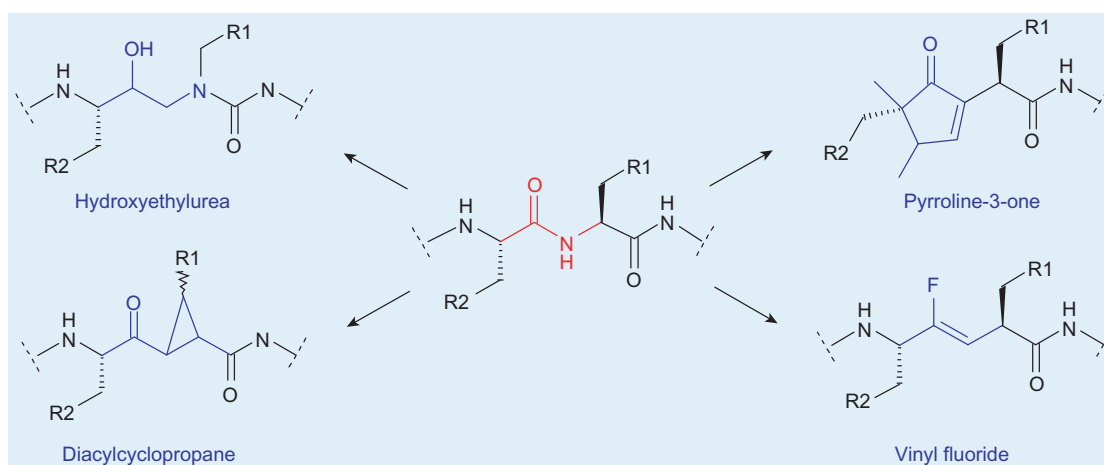


FIGURE 8.34 Unusual isosteric replacements for the peptidic bond.

TABLE 8.15 Heterocyclic Surrogates of the Amide Bond

	1,2,4-Oxadiazoles	β_3 adrenergic receptor agonist	Naylor et al. [152,153]; Feng et al. [154]; Biftu et al. [155]; Parmee et al. [156]
		Fatty acid oxidation inhibitors	Elzein et al. [157]; Koltun et al. [158]
	1,3,4-Oxadiazoles	Phe-Gly peptidomimetics NK1 receptor antagonists	Borg et al. [159]; Ladduwahetty et al. [160]
	Oxazoles	Fatty acid oxidation inhibitors	Elzein et al. [157]; Koltun et al. [158]
	Cyclic amidines	Dopamine D4 receptor agonists	Einsiedel et al. [161,162]
	Pyrroles	D3 antagonists	Einsiedel et al. [163]
	Phenylimidazoles	D4 receptor ligand	Thurkauf et al. [164]

In other cases, carboxamides are converted to sulfonamides, as illustrated by the synthesis of the hypoglycemic sulfonyl isostere of glybenclamide [147].

More unusual isosteric replacements for the peptidic bond were proposed and are reported in Figure 8.34. Among them, hydroxyethylureas were used in the design of a novel class of potent HIV-1 protease inhibitors, diacylcyclopropanes in the design of novel renin inhibitors, and pyrroline-3-ones for various proteolytic enzyme inhibitors [148,149]. Vinyl fluorides can probably be considered as representing the closest possible bioisosteres of the peptide bond. The synthetic methods available allow—by an appropriate selection of the precursors—the preparation of analogs of dipeptidic combinations of amino acids bearing no other functionalities in their side chains, for example, Gly, Ala, Val, Phe, and Pro [150]. Vinyl fluorides have been used in the design of bioisosteres of peptide bonds as in the case of the analgesic dipeptide 2, 6, -dimethyl-L-tyrosyl-D-alanine-phenylpropionamide [151].

Other structural modifications of the amide bond proposed to create more chemically stable and orally available molecules include heterocyclic rings, such as 1,2,4-oxadiazoles and 1,3,4-oxadiazoles (Table 8.15).

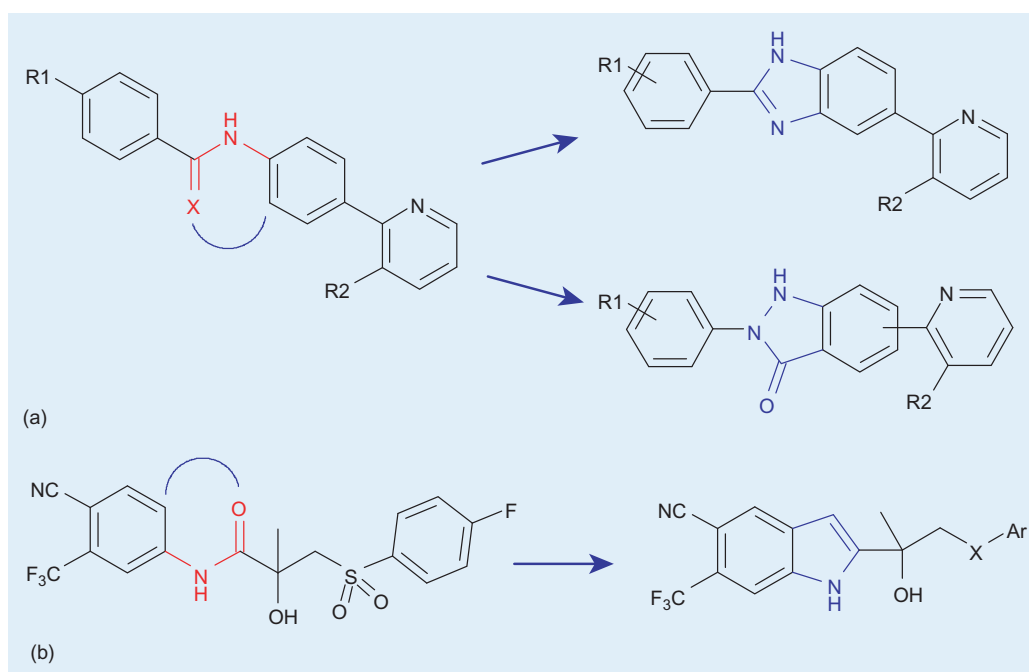


FIGURE 8.35 Benzimidazoles, indazolones, and indoles as carboxamides bioisosteres.

1,2,4-Oxadiazoles have been used as bioisosteres of the carboxamide moiety in SH2 inhibitors of tyrosine kinase ZAP-70 [165], 5-HT_{1D} receptor agonists [135], histamine H₃ receptor antagonists [166], and 5-HT₃ receptor antagonists [167]. An example worthy of note is given by the β_3 adrenergic receptor agonists, where the replacement of the amide bond with 1,2,4-oxadiazoles led to compounds with improved oral bioavailability retaining the β_3 AR agonist activity [152–156]. 1,3,4-Oxadiazoles have also been used as amide bond surrogates in several therapeutic areas like benzodiazepine receptor agonists [168], muscarinic receptor agonists [169], and NK1 receptor antagonists [160], and they have also been used as Phe-Gly peptidomimetics [159].

Other heterocyclic replacements of the amide bond include oxazole rings [157,158], cyclic amidines [161,162], pyrrole rings [163], and phenylimidazole [164] (Table 8.15).

In the case of aryl amides, heterocyclization was explored in order to overcome the pharmacokinetic liability. The search for transient receptor potential vanilloid 1 (TRPV1) antagonists as potential analgesic has led to the discovery of a series of biaryl amides (such as those reported in Figure 8.35a) that suffer from a pharmacokinetic liability [170]. In order to overcome this problem, heterocyclization was explored and has yielded benzimidazoles and indazolones as promising carboxamides bioisosteres [170] (Figure 8.35a). Other examples of benzimidazoles as aryl amides isosteres has also been reported as melanin-concentrating hormone receptor 1 (MCH R1) antagonists [171,172].

The indole ring has also been reported as a valuable carboxamide bioisoster (Figure 8.35b) of the nonsteroidal antiandrogen bicalutamide [173].

4. Urea and Thiourea Bioisosteres

Chemical modifications of the thiourea group of the metamide led to the discovery of *N*-cyanoguanidine, *N*-nitro-ethene diamine, and *N*-aminosulfonyl guanidine as successful bioisosteres of the urea group [44] (Figure 8.19). This successful example of bioisosterism has been applied to the sulfonyl-urea function of toseamide [174], a diuretic loop (Figure 8.36). This investigation led to the design and synthesis of the corresponding thiourea, cyanoguanidine, and diaminonitroethylene group. The structure, the electronic features (charges, molecular electrostatic potential, molecular orbitals), and the lipophilic properties of these three analogs were determined. In conclusion, cyanoguanidine and diaminonitroethylene turned out to be valuable isosteres of the (thio) urea function, as they share common geometric and electronic properties. In addition, they cover a range of lipophilicity that makes them suitable for compound pharmacomodulation, as illustrated by the diuretic and antiepileptic properties of analogs of toseamide.

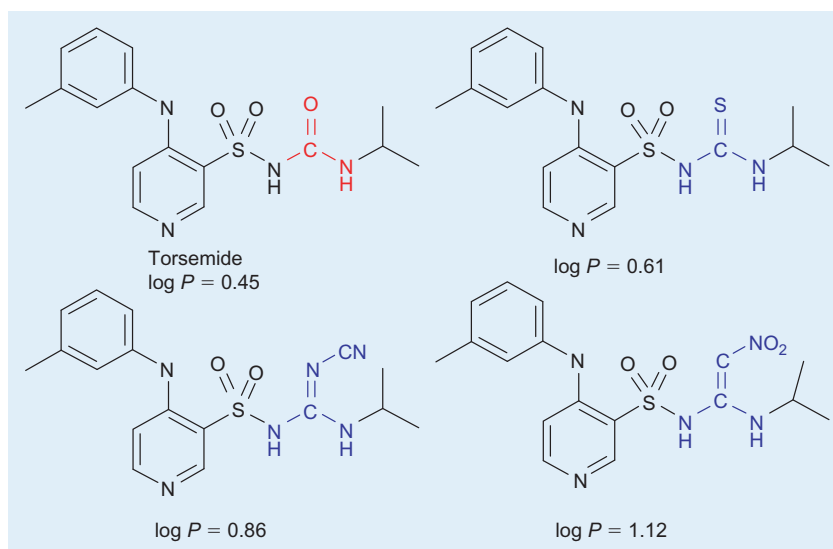


FIGURE 8.36 Structure of toseamide and its urea bioisosteres.

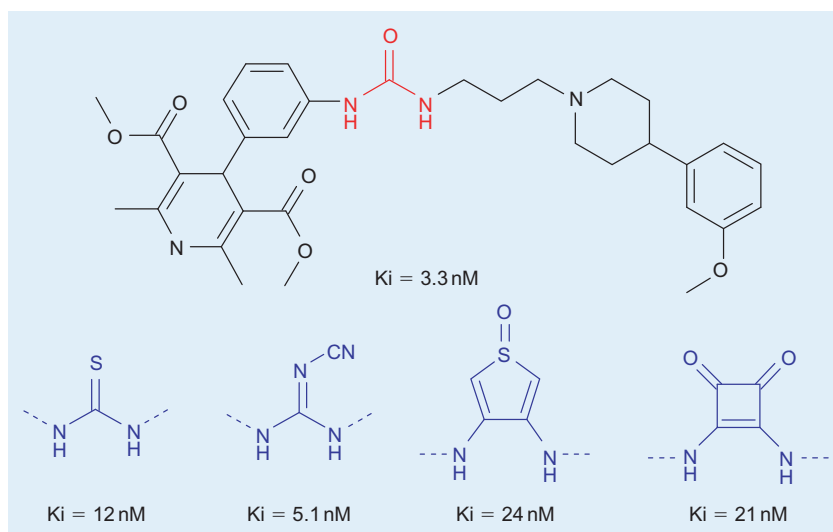


FIGURE 8.37 Binding affinities for various urea bioisosteres acting as antagonists at the NPY Y_1 receptor.

In a study undertaken on antagonists of the dihydropyridine neuropeptide Y_1 receptor, common bioisosteres such as thiourea and cyanoguanidine were examined as urea replacement (Figure 8.37) and also more uncommon derivatives like squaric acid and thiadiazole oxide [175,176]. Both cyanoguanidine and thiourea derivatives demonstrated potent binding affinity at the Y_1 receptor. The two heterocycle replacements, squaric acid and thiadiazole oxide, demonstrated good binding affinity, although both were ten times less active compared to parent urea.

Among more exotic surrogates, the 3,4-diamino thiadiazole dioxide moiety was proposed as a weakly acidic urea equivalent (Figure 8.38) [177]. The similar thiatriazole dioxide is found in the H_2 antagonist tuvatidine (HUK 978). Other bioisosteres are exo–endo amidinic heterocycles bearing an electron-attracting function in the α position [178,179] (Figure 8.38).

5. Bioisosteres of the Phenol Function

The optimal bioisosteres of the phenolic function should have approximately the same size as the hydroxyl itself and should have approximately the same acidity range (weak acid) and be able to form hydrogen bonds.

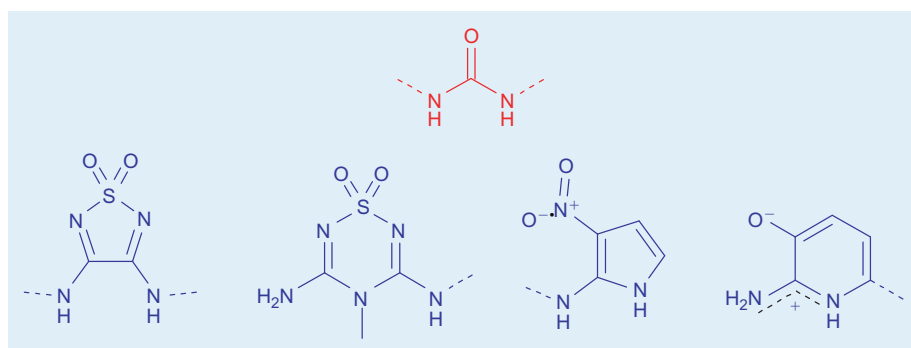


FIGURE 8.38 Less common urea equivalents.

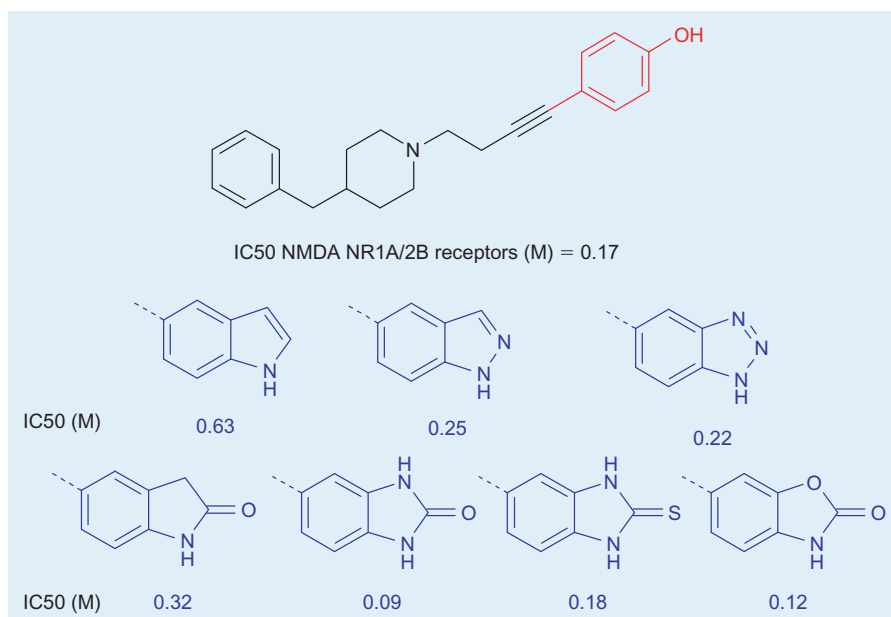


FIGURE 8.39 Bioisosteric replacements of the phenol function in the design of *N*-methyl-D-aspartate (NMDA) receptor antagonists.

The most popular surrogates for phenolic functions are NH groups rendered acidic through the presence of an electron-attracting group. Figure 8.39 shows an application of this bioisostery in the design of *N*-methyl-D-aspartate (NMDA) receptor antagonists [180]. In this case, the replacement of the phenol by heterocyclic NH-containing rings was performed in order to slow metabolism and hence to improve oral bioavailability. Indeed, the potent and NR1A/2B-receptor selective benzimidazolone analog was obtained, demonstrating oral activity in a rodent model of Parkinson's disease at 10 and 30 mg/kg.

Using this analogy, Wu et al. [181] prepared a series of phenolic bioisosteres of benzazepine D₁/D₅ antagonists (Figure 8.40).

Compared to the reference compound SCH 38393, they exhibit similar or more potent activities, high selectivity over D₂–D₄ receptors, and improved *in vivo* pharmacokinetics. The optimization of the hydrogen bond donating capacity of various heterocycles allowed the identification of several potent D₁/D₅ antagonists with high selectivity D₂–D₄, α_{2a} adrenergic receptors, and the 5-HT transporter, keeping at the same time excellent pharmacokinetic profiles.

Other popular phenol bioisosteric substituents [182,183] include methanesulfamide (CH₃SO₂NH–), hydroxymethyl (HOCH₂–) or hydroxyisopropyl (HOC(CH₃)₂–), various amide groups (–NHCHO, –NHCOCH₃, –NHCOC₆H₅),

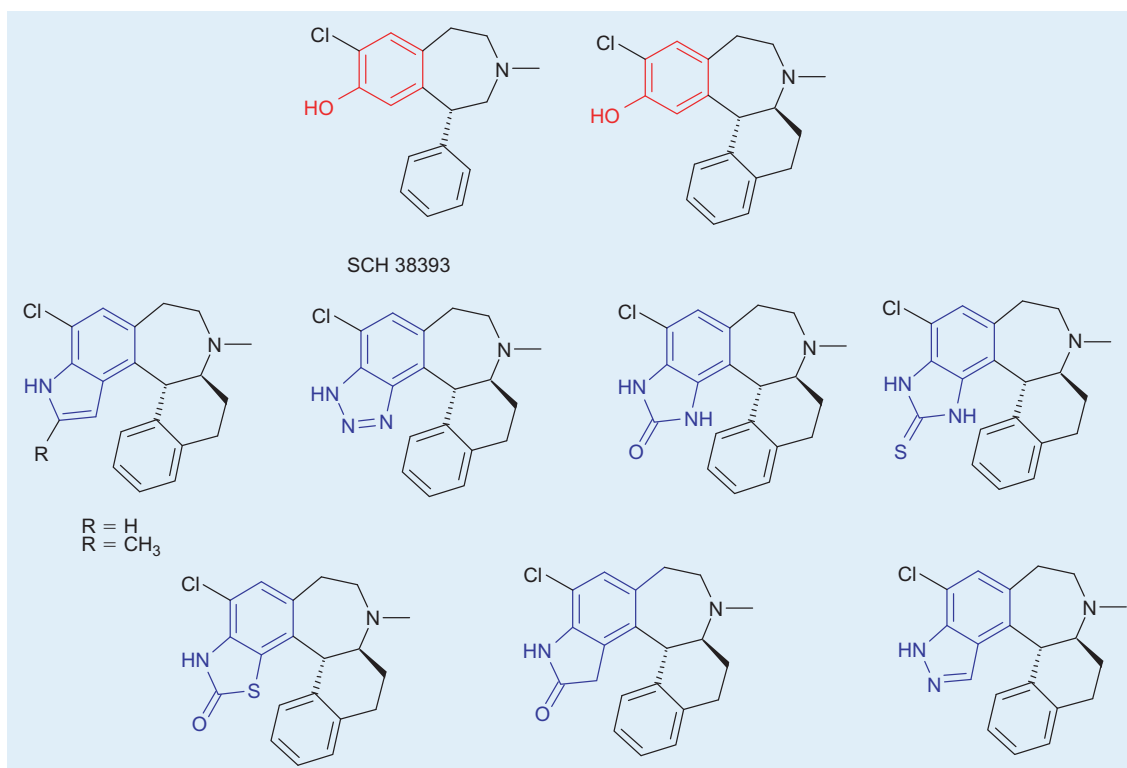


FIGURE 8.40 Phenol bioisosteres of benzazepine D₁/D₅ antagonists.

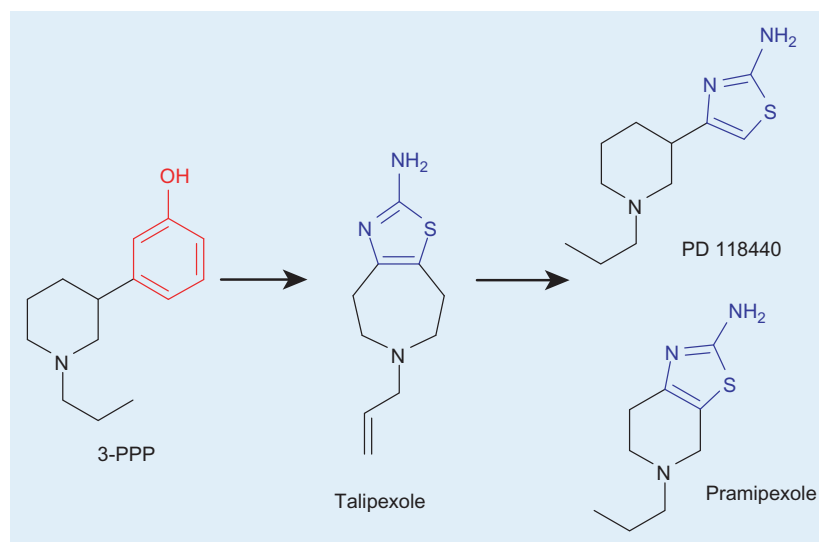


FIGURE 8.41 2-Aminothiazole bioisostery applied to the dopamine agonist 3-PPP.

methanesulfamidomethyl ($\text{CH}_3\text{SO}_2\text{NHCH}_2-$), dimethylaminosulfonamide ($(\text{CH}_3)_2\text{NSO}_2\text{NH}-$), and others groups with an ionizeable proton next to or near an aromatic ring.

Hacksell et al. [184] described the compound $(\pm)$ -3-(1-propyl-3-piperidyl)phenol [$(\pm)$ -3-PPP] as highly selective for presynaptic brain DA receptors. However, the clinical potential of these molecules (Figure 8.41) as antischizophrenic or antiparkinsonian agents may be limited by their relatively low oral bioavailability and their short duration of action [185]. Thus, a heterocyclic analog of 3-PPP might retain some of its pharmacological

II. LEAD COMPOUND DISCOVERY STRATEGIES

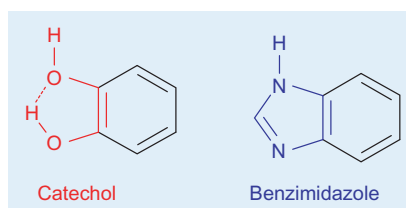


FIGURE 8.42 The bioisostery catechol/benzimidazole.

Compounds	Name	IC ₅₀ Values [μM]			
		IGF-1R	IR	SRC	PKB
	AG 538	0.06	0.12	2	76
	GB 19	0.37	0.6	1.5	21
	AGL 2263	0.43	0.4	2.2	55

FIGURE 8.43 Inhibition of IGF-1R and other kinases by AG 538 bioisosteres and analogs.

activity while having improved oral bioavailability and duration of action [186]. In the case of talipexole (B-HT 920) (Figure 8.41), a DA agonist that has been reported to be selective for DA autoreceptor, 2-aminothiazolyl moiety was used as a replacement for the phenol ring.

This successful example led successively to the discovery of PD 118440, a dopamine-autoreceptor agonist [186], and pramipexole, a DA agonist with preference for the DA D₃ (over DA D₂) receptor [187–189].

6. Catechol Bioisosteres

All the previous examples can be applied to the catechol family. Many of these bioisosteres share with the catechols the ability to chelate metal atoms and to form hydrogen-bonded second rings. In benzimidazole, this H-bond ring is mimicked by way of a covalent ring structure (Figure 8.42).

A recent case described the synthesis of more stable bioisosteres of inhibitors of the insulin-like growth factor-1 receptor kinase (Figure 8.43). Based on the structure of AG 538 [190], which contains two catechol rings and is sensitive to oxidation in cell, a new series of kinase inhibitors were developed. The catechol moiety was replaced by a benzoxazolone ring resistant to oxidation yielding two compounds, GB19 and AGL2263, which maintain the same potency as AG 538.

Until now, 2-aminothiazole derivatives or other aromatic heterocyclic systems have been used as a bioisosteric surrogate for the catechol nucleus (cf. studies around the pramipexole) [187]. Hübner et al. [191–193] evaluated nonaromatic catechol bioisosteres (Figure 8.44). They were able to demonstrate that the π -electronic system of the nonaromatic endiyne FAUC 88 and 73 can be used as an efficient bioisostere for the catechol fragment of dopamine. Combined with conformational rigidization, this approach led to dopamine-receptor agonists with high affinity for the subtypes of D₂-family and especially for D₃.

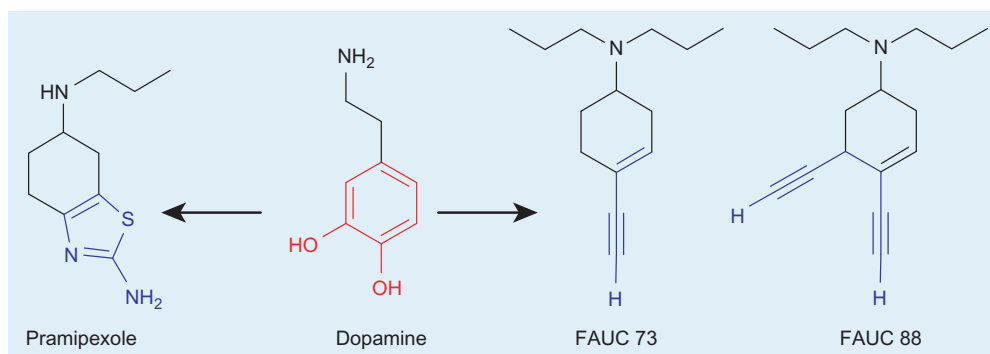


FIGURE 8.44 Aromatic and non-aromatic dopamine isosteres.

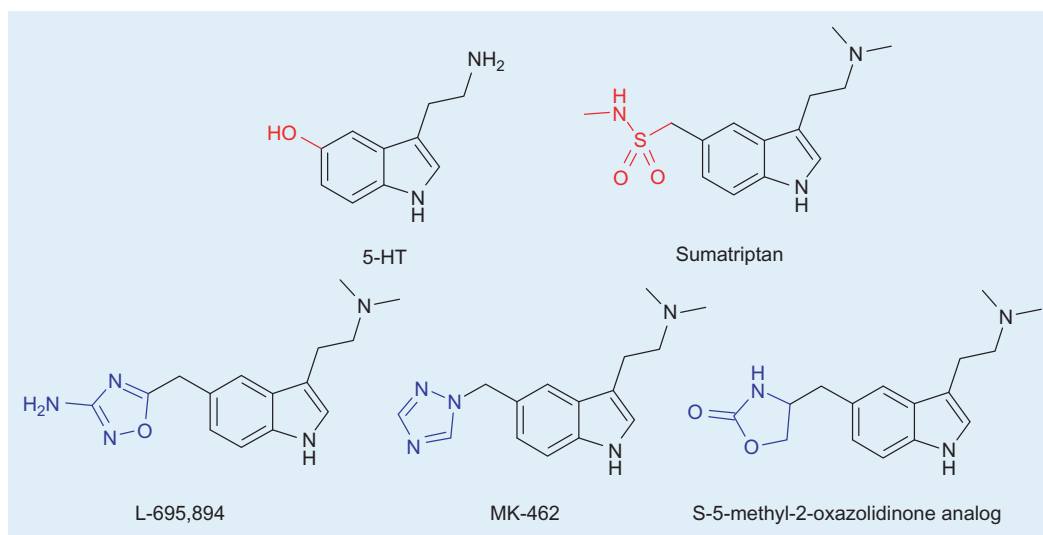
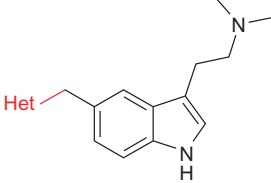


FIGURE 8.45 Replacement of the phenolic function of serotonin by various functions able to serve as H-bond acceptors.

7. Sulfonamides Bioisosteres

The introduction of the 5-HT_{1D} receptor agonist sumatriptan for the acute treatment of migraine has marked an intense research effort to discover more potent and selective 5-HT_{1D} receptor agonists with improved pharmacokinetic profiles [194]. The H-bond acceptor ability of the 5-position is crucial for 5-HT_{1D} receptor affinity and selectivity as was revealed by reference compounds (5-HT and sumatriptan). The search for other analogs with better bioavailability led to the replacement of the methyl sulfonamide with different heterocycles that are able to be H-bond acceptors. The amino-oxadiazole analog, L-695,894 (Figure 8.45), has a better oral bioavailability but a lack of good selectivity. In fact, it has a significant affinity for 5-HT_{2A} and 5-HT_{2C} receptors [135]. Alternative five-membered heteroaromatic rings were explored to find analogs with log Ds < -0.5 in order to minimize the central nervous system penetration [194]. Simple unsubstituted imidazoles, triazoles, and tetrazoles (Table 8.16) have high affinity and good selectivity for the 5-HT_{1D} receptor. The 1,2,4-triazole 1, MK-462, was shown to have the optimal pharmacokinetic profile with rapid oral absorption and high bioavailability.

Using a different approach, Glen et al. [195] built a pharmacophore based on the pharmacological activities of a series of novel C- and N-linked hydantoin analogs. Their model was used as a framework for the design of a diverse series of analogs with good affinity and selectivity for 5-HT_{1D}. Suitable with the constraints required for good oral absorption, a potent selective 5-HT_{1D} agonist, S-5-methyl-2-oxazolidinone analog, has been described.

TABLE 8.16 Displacement of [3H]-5-HT to 5-HT1D Recognition Sites in Pig Caudate Membranes by N-Linked Imidazoles, Triazoles and Tetrazoles and Standard 5-HT1D Agonists


Compound	Het	pIC ₅₀	log D ^a
5-HT		8.0	
Sumatriptan		7.7	− 1.17
L-695,894		7.6	− 0.67
1, MK-462		7.3	− 0.74
2		7.5	− 0.53
3		7.2	− 0.74
4		7.3	− 0.70
5		6.6	− 0.20
6		7.4	− 0.64
7		7.4	− 0.34

^alog P measured at pH 7.4.

The dipolar azido group is a bioisostere [196] of the SO₂NH₂ and SO₂Me hydrogen bonding pharmacophores present in many selective COX-2 inhibitors, and the azido analogs **14** and **15** are useful biochemical agents for photoaffinity labeling of the COX-2 enzyme (Figure 8.46).

F. Reversal of Functional Groups

The reversal of the peptidic functional groups is often used in peptide chemistry. The obtained retropeptides are generally more resistant to enzymatic attacks (Figure 8.33) [146,197]. For thiorphan and *retro*-thiorphan, an identical binding mode to the zinc protease thermolysin was demonstrated [198]. Similar inhibition values for thermolysin and neutral endopeptidase were observed, whereas, for another zinc protease, angiotensin-converting enzyme (ACE), noticeable differences for inhibition were found (Figure 8.47).

But the strategy of functional inversion can also be applied to nonpeptidic compounds. A historical example is the change from orthoform to neo-orthoform (orthocaine; Figure 8.48). The unwanted side effects often encountered with aromatic para-amino substituted compounds (“para effects,” essentially of allergic origin) are abolished in the meta amino isomer, whereas the local anesthetic activity is maintained. Similarly the “meta” isomer of benoxinate has a local anesthetic activity identical to that benoxinate itself [199].

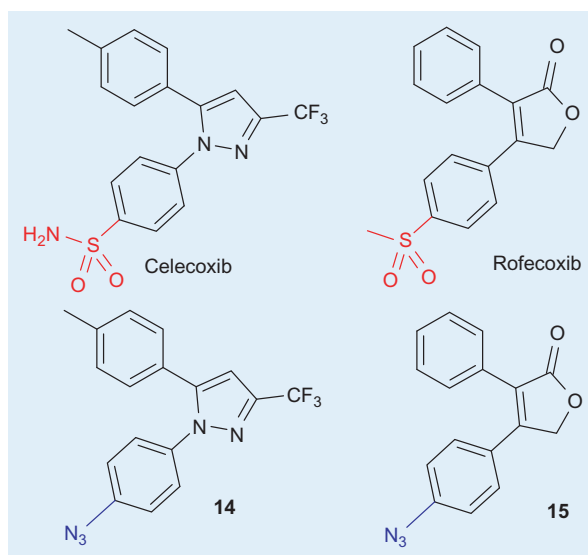


FIGURE 8.46 Selective COX-2 inhibitors and their azido bioisosteres.

	Enzyme	K _i value in M
 Thiorphan	NEP 24.11	0.0019
	Thermolysin	1.8
	ACE	0.14
	Enzyme	K _i value in M
 Retro-thiorphan	NEP 24.11	0.0023
	Thermolysin	2.3
	ACE	>10

FIGURE 8.47 Inhibition values of thiorphan and *retro*-thiorphan for three zinc proteases.

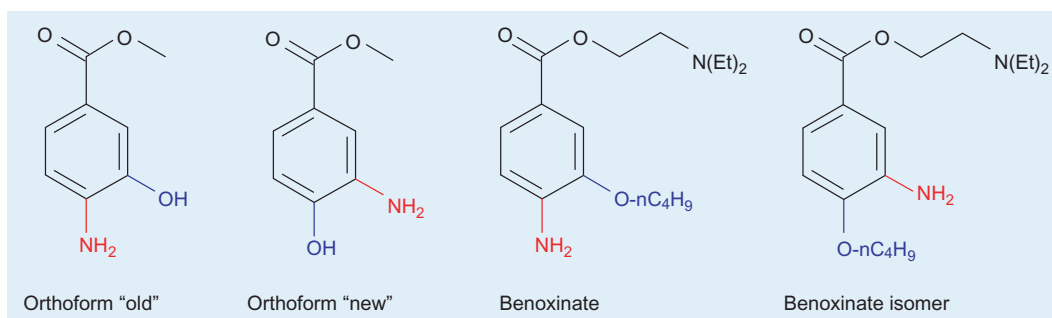


FIGURE 8.48 Positional isomery in local anesthetics [199].

II. LEAD COMPOUND DISCOVERY STRATEGIES

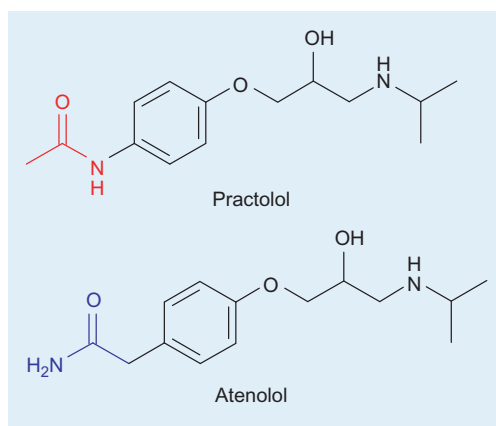


FIGURE 8.49 Reversal of functional groups in practolol yields atenolol.

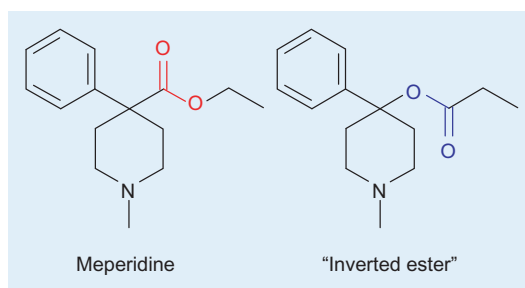


FIGURE 8.50 Meperidine and the corresponding inverted ester [17].

The β -blocking agent practolol, which was one of the first cardioselective β -blockers, was rapidly replaced by its isomeric analog atenolol, which presents fewer side effects (Figure 8.49).

The inversion of the ester function of *meperidine* leads to 1-methyl-4-phenyl-4-propionyloxy piperidine (Figure 8.50), which is five times more potent as an analgesic drug than meperidine and represents the model compound of the series of inverted esters [17].

The change from indomethacin to clometacin, although representing a clean example of functional group reversal, causes more profound alterations than that shown in the previous examples (Figure 8.51). At a first glance, this change can even seem too drastic; however, in rotating the molecule of clometacin by 180°, the resemblance with the parent molecule becomes evident.

Indomethacin is mainly used as a nonsteroidal anti-inflammatory agent and occasionally as an analgesic; clometacin on the other hand is usually recommended as an analgesic and shows weak anti-inflammatory properties. Applied to serotonin, a similar reversal of a functional moiety yielded 5HT_{2C} receptor selective agonists [200] (Figure 8.52).

IV. SCAFFOLD HOPPING

Scaffold hopping is a useful technique used by medicinal chemists to discover structurally novel compounds starting from known active molecules by changing the molecular backbone of the template. Medicinal chemists quite often have the need of “jumping” in a different chemical space: new chemical entities offer the choice of easier chemical accessibility and so possibility of speeding up the lead optimization process. Switching to a different molecular backbone can help avoid some undesirable ADME-tox properties. Scaffold hopping also comes to a rescue to move from complex natural molecules to easier synthesizable

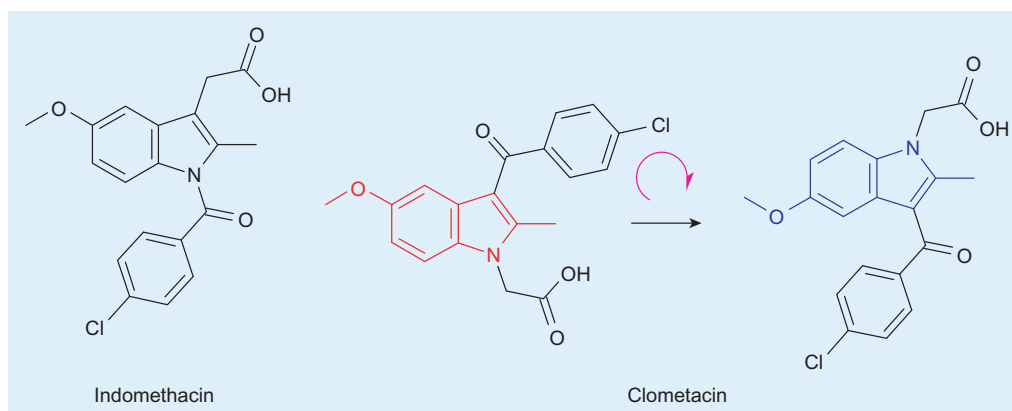


FIGURE 8.51 Functional inversion applied to indomethacin.

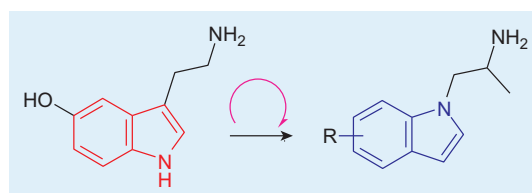


FIGURE 8.52 Serotonin analogs resulting from a functional reversal [200].

small molecules. Moreover “jumping” in a new chemical series isofunctional with a given one is fundamental when new IP position is sought.

A. Successful Examples of Serendipitous Scaffold Hopping

The concept of scaffold hopping relies upon the assumption that structurally and chemically distinct templates interact with the same receptor, inducing equivalent biological activity. This seems to contradict the leading principle of the lead optimization process and drug design that is based on the concept that chemical similarity is reflected by similar biological activity. On the other hand, this concept is not always true. Structural and chemical analogy does not always predict similar biological affinity, as it is shown in Figure 8.53. In fact, promethazine, imipramine, and chlorpromazine, which are strictly similar from a structural point of view, interact with different receptors and present completely different therapeutic profiles acting respectively as H1 antagonist, uptake inhibitor, and dopamine antagonist.

Indeed scaffold hopping can be interpreted as the broadest expansion of the bioisosterism concept. The idea of scaffold hopping is indeed not new; it is rather a new term to designate known experimental evidences. A retrospective analysis of the marketed drugs and/or of the ligands acting on the same receptors brings up some interesting examples of structurally different molecules eliciting the same biological activity. A nice example is given by the nonsteroidal anti-inflammatory drugs (NSAIDs) COX-2 inhibitors, as illustrated in Figure 8.54, which lists four known traditional NSAIDs: indomethacin, nimesulide, diclofenac, and celecoxib.

Actually, the GABA-A ligands at the benzodiazepine site give a nice picture of matching and mismatching of scaffold hopping and bioisosterism (Figure 8.55). Looking at diazepam, zolpidem, zopiclone, and zaleplon, a structural analogy is barely found. Despite this, they are all GABA-A ligands acting as full agonists. The same can be said about the inverse agonists DMCM, 16, 17, FG 8094, and 18: chemically different compounds have comparable biological activity. On the other hand, similar compounds like FG 8094, Bretazenil, and Ro 15-1788 do not give the same biological response.

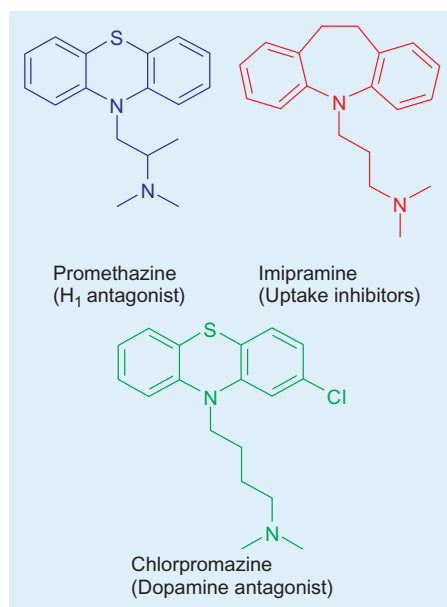


FIGURE 8.53 Structure and chemical similarity are not always synonymous of similar biologically active compounds.

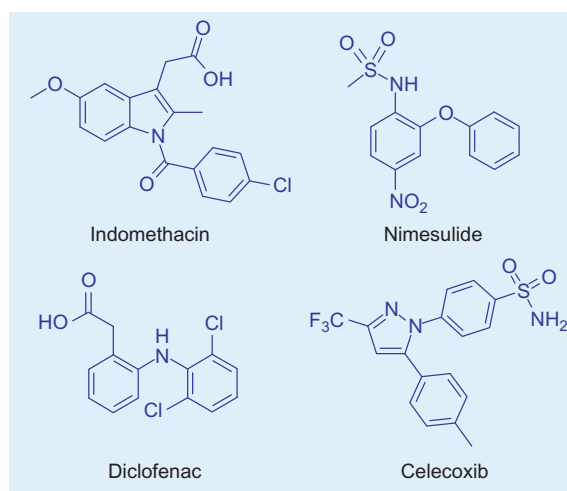


FIGURE 8.54 An example of scaffold hopping: four structurally different molecules have similar therapeutic properties by interacting with the same receptor.

B. Scaffold Hopping and Virtual Screening

The examples cited above illustrate that similar biological activity can be obtained with structurally different molecules, which seems to contradict the bioisosterism principle discussed in this chapter. The serendipitous examples of scaffold hopping described above have been generated either by high-throughput screening (HTS) or by selective optimization of side activity (the SOSA approach) [201]. In other studies, the “hopping” in a different molecular scaffold has relied upon the intuition and/or the experience of the medicinal chemist working on the project. Most of the virtual screening studies aiming to hop in isofunctional molecules are based on the bioisosterism concept that structural likeness predicts analogous biological activity. At a first glance, it can be concluded that a rational approach to scaffold hopping is not possible. Indeed molecular modeling and

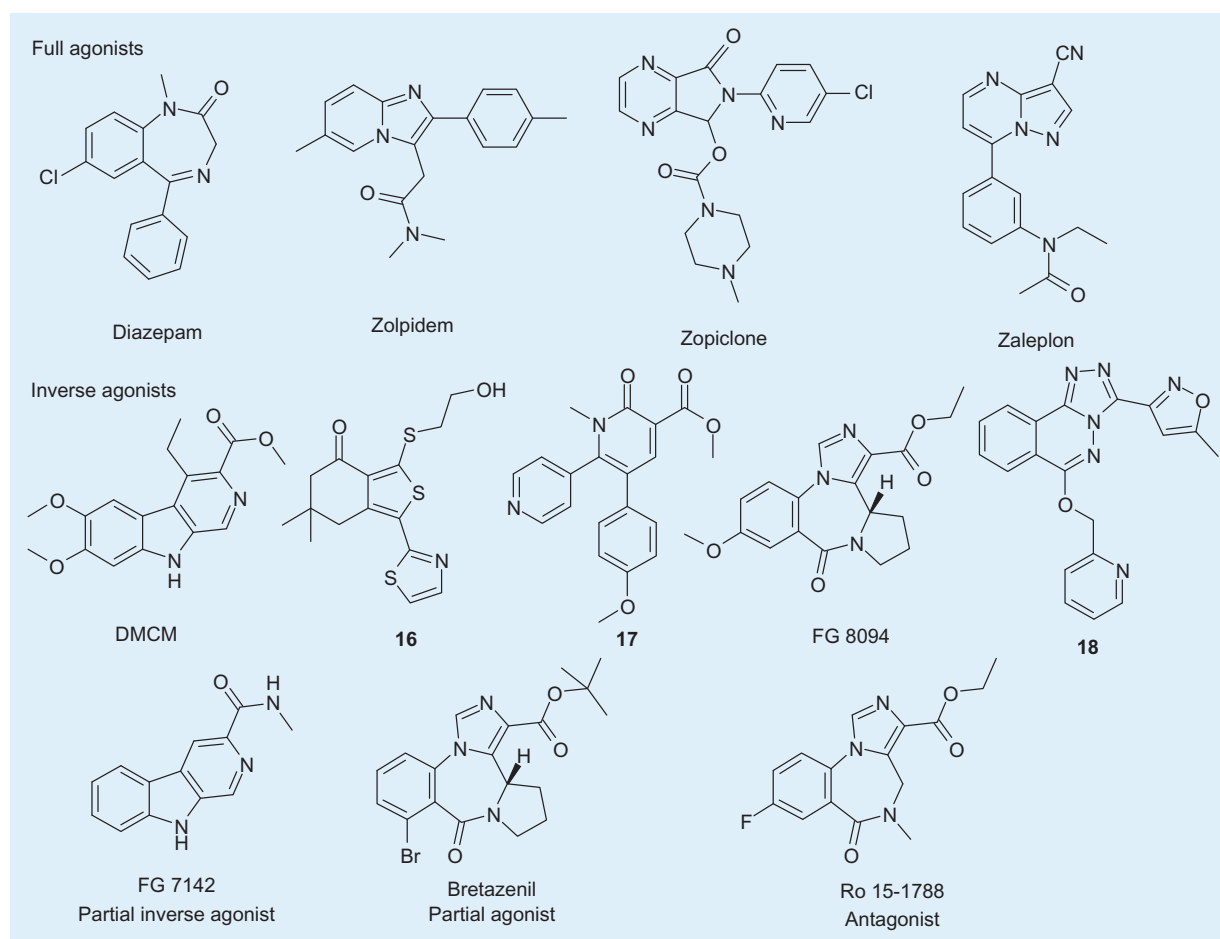


FIGURE 8.55 GABA-A ligands binding at benzodiazepine site.

chemoinformatics experts have set up a lot of software and methods to address the paradox of scaffold hopping and bioisosterism to find a logical process [202,203].

Among the virtual screening techniques reported so far, four types of computational approaches for scaffold hopping can be distinguished: (a) shape matching, (b) pharmacophore searching, (c) fragment replacement, and (d) similarity searching. Most of the programs offer combinations of several approaches, and they are used—according to the case scenario of a given project—either as on a ligand-based virtual screening base or on a structure (receptor)-based screening. Other studies have reported a combination of the two methods to increase the performance of the virtual screening. In a case where more active ligands are known but no information is known about the receptor structure, it will be more useful to proceed through shape matching combined with a pharmacophore searching. By contrast, when the receptor structure is known, a virtual screening based on molecular docking is a powerful and successful technique. A detailed description of the computational approaches of scaffold hopping goes beyond the scope of this chapter and only a few illustrative examples will be reported. Figure 8.56 reports an example of scaffold hopping related to the design of a novel cholecystokinin-2 (CCK₂) antagonist [204]. Starting from a known series of indoles (19) with high *in vitro* affinity and selectivity but poor bioavailability, a CCK₂ pharmacophore receptor has been identified and used to design new scaffolds to overcome the problems associated with the high level of biliary elimination of the compound 19. Two new series of pyrroles and imidazoles (20) were identified, and although they are not as potent as the original indole derivative *in vitro*, they have greater potential as drug candidates because of their reduced tendency to biliary excretion.

Scaffold hopping is an extremely useful technique to switch from peptide ligands to small molecule ligands. A fragment-based docking procedure followed by a substructure search were used to identify a set of small

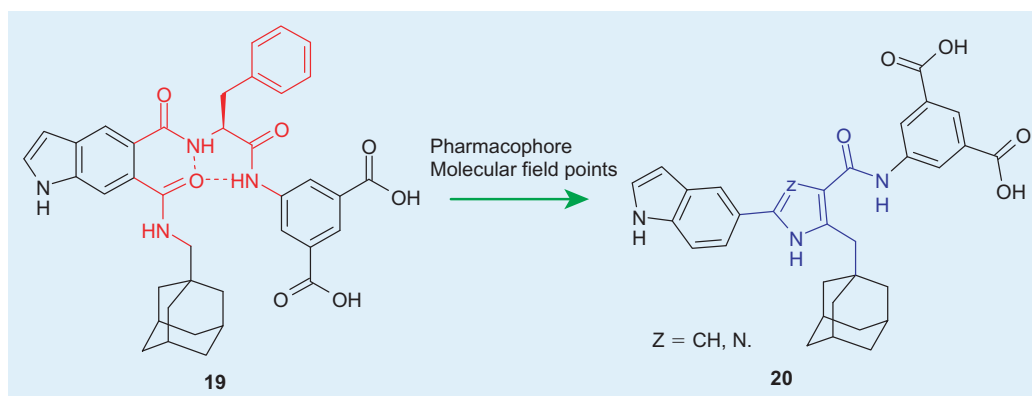


FIGURE 8.56 Design of a novel CCK₂ antagonist via a scaffold hopping approach from a known series of indoles.

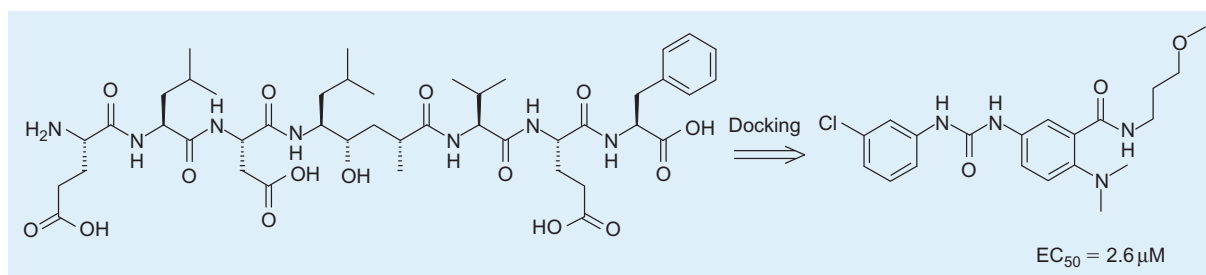


FIGURE 8.57 Discovery of non-peptide inhibitor of β -secretase by high-throughput docking.

molecules as β -secretase inhibitors [205]. Figure 8.57 shows one ligand identified with virtual screening that may serve as a starting point for further optimization for Alzheimer's disease.

Most of the studies based on virtual screening use a hierarchical approach combining several conceptually different techniques [206]. The most common combination used is a 3D pharmacophore modeling followed by a docking study. Figure 8.58 contains an illustrative example of this combined use: a set of ten known inhibitors of Human Rhinovirus Coat protein was used to design a 3D pharmacophore that was subsequently used for a docking study of the Maybridge DB [206].

A set of ten compounds was retrieved according to the score fit of the molecules. The ten molecules were tested *in vitro*, and all of them displayed antirhinoviral activity in the micromolar range. Particularly promising results were achieved with the compound reported in Figure 8.58, which shows an EC_{50} of 4.3 μM .

V. ANALYSIS OF THE MODIFICATIONS RESULTING FROM ISOSTERISM

It is rare that the replacement of a part of a molecule by an isosteric or bioisosteric group leads to a strictly identical active principle. In practice, that is not even sought, and one prefers that the new compound produces a change as compared to the parent molecule. In general, the isosteric replacement, even though it represents a subtle structural change, results in a modified profile: some properties of the parent molecule will remain unaltered and others will be changed. Bioisosterism will be productive if it increases the potency, the selectivity, and the bioavailability, or decreases the toxicity and undesirable effects of the compound. In proceeding to isosteric modifications, one will focus predominantly on a given parameter (e.g., structural, electronic, hydrophilic), but it is all but impossible not to alter several parameters simultaneously.

II. LEAD COMPOUND DISCOVERY STRATEGIES

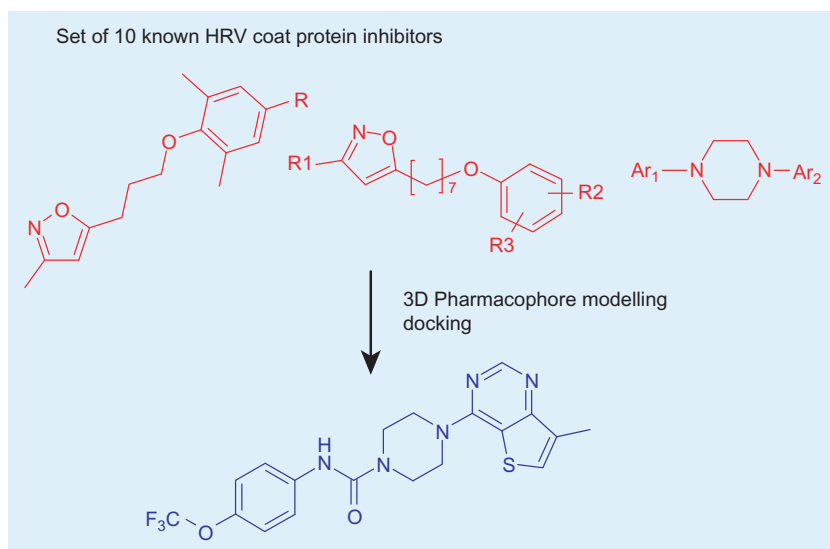


FIGURE 8.58 A set of ten known HRV coat protein inhibitors was used for 3D pharmacophore modeling followed by docking study afforded new candidate structure suitable for an optimization process.

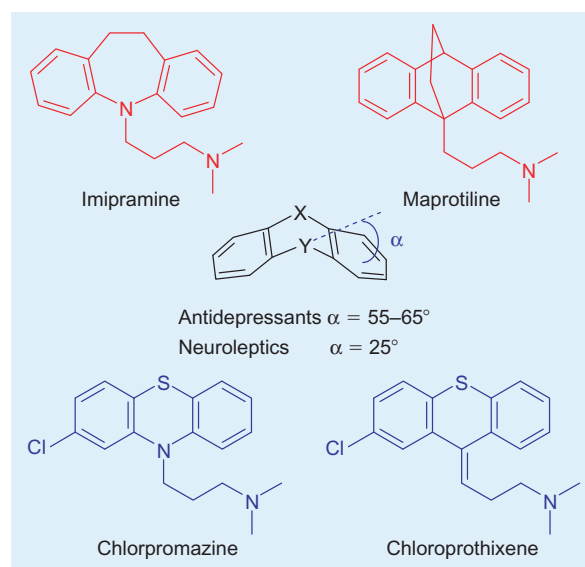


FIGURE 8.59 The tricyclic antidepressants (imipramine and maprotiline) are characterized by an dihedral angle of 55° to 65° between the two benzo rings, this angle is only 25° for the tricyclic neuroleptics (chlorpromazine, chlorprothixene) [207].

A. Structural Parameters

These will be important when the portion of the molecule involved in the isosteric change serves to maintain other functions in a particular geometry. That is the case for tricyclic psychotropic drugs (Figure 8.59).

In the two antidepressants (imipramine and maprotiline), the bioisosterism is geometrical insofar as the dihedral angle α formed by the two benzo rings is comparable: $\alpha = 65^\circ$ for the dibenzazepine and $\alpha = 55^\circ$ for the dibenzocycloheptadiene [207]. This same angle is only 25° for the neuroleptic phenothiazines and the thioxanthenes. In these examples, the part of the molecule modified by isosterism is not involved in the interaction with the receptor. It serves only to position the other elements of the molecule correctly.

The structure of various bioisosteric retinoic acid receptor agonists highlights the dominantly geometric parameter of this bioisostery (Figure 8.60) [208].

II. LEAD COMPOUND DISCOVERY STRATEGIES

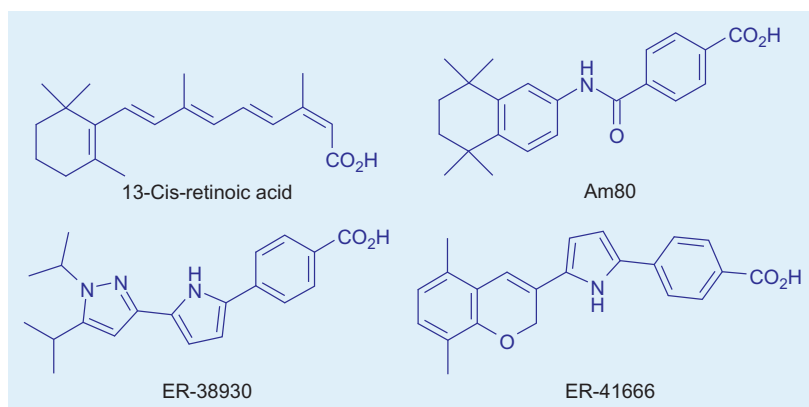


FIGURE 8.60 Bioisostery in its broadest sense.

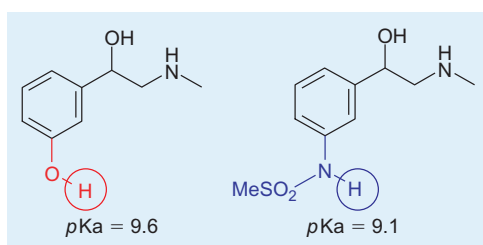


FIGURE 8.61 An example of bioisosterism, or nonclassical isosterism, the methanesulfonamide substituent has comparable acidity to the phenolic hydroxyl group [197].

B. Electronic Parameters

Electronic parameters govern the nature and the quality of ligand–receptor or ligand–enzyme interactions. The relevant parameters will be inductive or mesomeric effects, polarizability, pK_a , capacity to form hydrogen bonds, etc. Despite their very different substituents in the *meta* position, the two epinephrine analogs (Figure 8.61) exert comparable biological effects: they are both β -adrenergic agonists. In fact, the key parameter resides in the very close pK_a values [197].

C. Solubility Parameters

When the functional group involved in the isosteric change plays a role in the absorption, the distribution, or the excretion of the active molecule, the hydrophilic–lipophilic parameters become important.

Imagine in an active molecule the replacement of $-\text{CF}_3$ ($\pi = +0.88$) by $-\text{CN}$ ($\pi = -0.57$) (Figure 8.62). The electron-attracting effect of the two groups will be comparable, but the molecule with the cyano function will clearly be more hydrophilic. This loss in lipophilicity can then be corrected by attaching elsewhere on the molecule a propyl, isopropyl, or cyclopropyl group.

D. Anomalies in Isosterism

In this section, two applications of the bioisosterism concept that imply unusual behaviors of commonly encountered atoms or groups are discussed.

1. Fluorine–Hydrogen Isosterism

It is an anomaly that fluorine does not resemble other halogens—notably chlorine—while on the other hand it often mimics an atom of hydrogen [209].

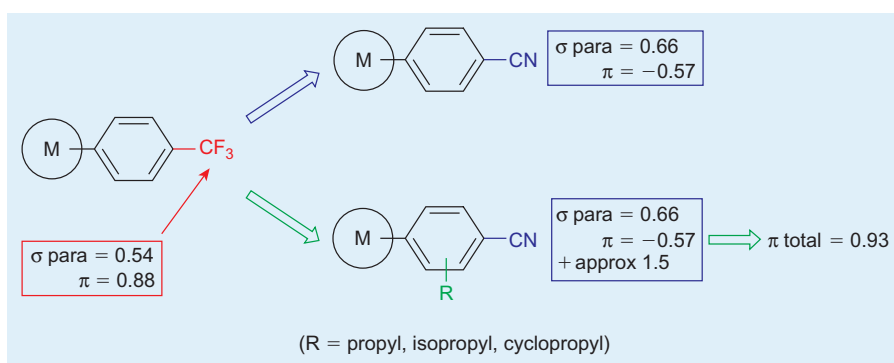


FIGURE 8.62 The loss in lipophilicity resulting from the bioisosteric exchange of a CF_3 for a CN has to be compensated by the equivalent of a three carbon residue.

TABLE 8.17 Fluorine–Hydrogen Isosterism. Observe the Comparable Sizes of the Two Atoms, whereas Chlorine is Close to the Methyl and Trifluoromethyl

Parameter	H	F	Cl	CH_3	CF_3
Atomic radius	0.29	0.64	0.99	—	—
Van der Waals radius	1.2	1.35	1.80	<2	<2
Molecular refractivity	1.03	0.92	6.03	5.65	5.02
Electronic effect (<i>para</i> σ) ^a	0.00	0.06	0.23	− 0.17	0.54
Resonance effect (<i>R</i>) ^a	0.00	− 0.34	− 0.15	− 0.13	0.19
Electronic effect (σ^*) ^b	—	3.08	2.68	0.00	2.85

^aFor aromatic systems.

^bfor aliphatic systems.

1. **Steric aspects:** The fluorine atom is considerably smaller than the rest of the halogen atoms. Seen from the steric point of view, it resembles more hydrogen than chlorine (Table 8.17). Effectively, fluoro-derivatives differ from the other halogenated derivatives because fluorine forms particularly stable bonds with carbon and—in contrast to other halogens—is only rarely ionized or displaced. Because it is both chemically inert and of small size, organic fluorine is often compared to hydrogen.

One understands especially the incorporation by living organisms of fluoroacetic acid in place of acetic [210] acid or of 5-fluoro-nicotinic acid and 5-fluoro-uracil as antimetabolites. This “fraudulent” incorporation leads to lethal syntheses [211]. This is generally not the case with the corresponding chlorinated, brominated, or iodinated analogs.

2. **Electronic aspects:** Fluorine is the most electronegative of the halogens (Table 8.17) and forms particularly stable bonds with carbon atoms. This chemical inertia explains why fluoro-derivatives are more resistant to metabolic degradation (Figure 8.63). Thus, for the β -haloalkylamines (nitrogen mustards), the alkylating activity is lost when chlorine or bromine are replaced by fluorine or by hydrogen [212].

The isosterism between H and F will therefore often serve to give analogs that are more resistant to metabolic degradation (obstructive halogenation: flunarizine and in flufenisal; Figure 8.63). Similarly the CF_3 group is biostable, whereas CH_3 is easily oxidized [209].

3. **Absence of *d* orbitals:** Another difference between fluorine and the other halogens comes from the absence of a *d* orbital for fluorine, and thus its incapacity to participate in resonance effects with a donor of π electrons (Figure 8.64).

This explains why *para*-fluorophenol is slightly less acidic than phenol, while for other *para*-halogenated phenols the acidity changes in parallel with the atomic number (Table 8.18 [209]).

4. **Case study:** A good example of continuous variation of activity in halogenated compounds is provided by a series of antihistaminic drugs related to tripeleminamine (Figure 8.65; X = H).

II. LEAD COMPOUND DISCOVERY STRATEGIES

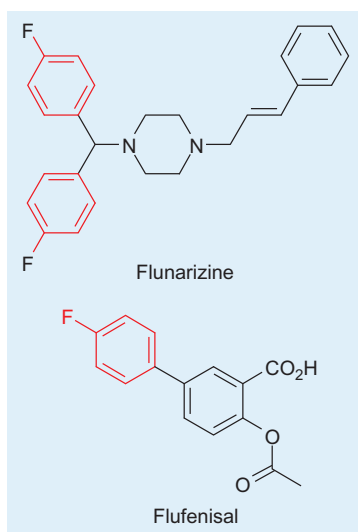


FIGURE 8.63 In flunarizine and in flufenisal fluorine atoms in para position prevent metabolic hydroxylation.

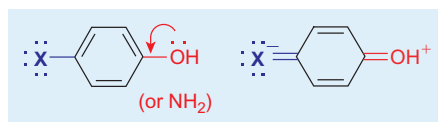


FIGURE 8.64 The resonance between the OH lone pair and the X group is not possible if $\text{X} = \text{F}$.

TABLE 8.18 Dissociation Constants of *p*-Halogenated Phenols [209]

Compound	Dissociation constant $K_a \times 10^3$ [210]
Phenol	0.32
<i>p</i> -Fluorophenol	0.26
<i>p</i> -Chlorophenol	1.32
<i>p</i> -Bromophenol	1.55
<i>p</i> -Iodophenol	2.19

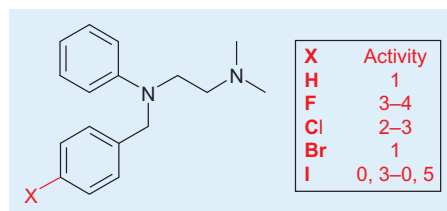


FIGURE 8.65 Variation of activity in a series of antihistaminic compounds as a function of the halogenated para-substituent [213].

II. LEAD COMPOUND DISCOVERY STRATEGIES

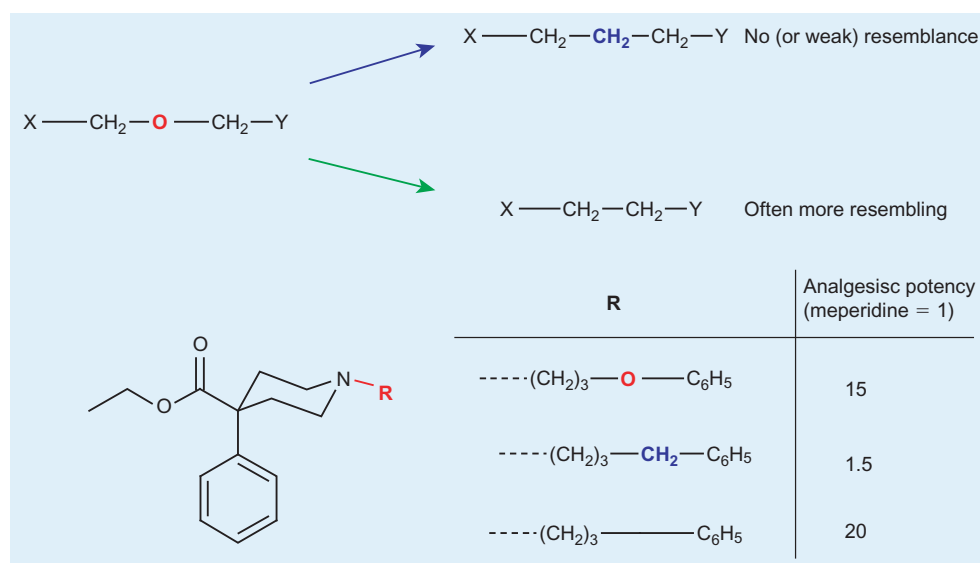


FIGURE 8.66 Friedman's ether oxygen-methylene group paradox [2].

Apparently we are dealing here with a classical isosteric series: F, Cl, Br, I, but one sensitive to steric hindrance in the para position. Probably what happens *in vivo* is para-hydroxylation of the benzene ring. The best candidate becomes then the para-fluoro compound, since it is not bulkier than the unsubstituted compound, while being biostable.

2. Exchange of Ether Oxygen and Methylene Group

Ether oxygen atoms and methylene groups possess a similar tetrahedral structure and should normally be isosteric. In fact, the isosterism between O and CH₂ very often yields anomalous results, which brought Friedman [214] to the interesting observation "that the omission of the ether oxygen changes biological activity much less in some cases than the replacement by the isosteric methylene group" (Figure 8.66). In the meperidine series, for example, the change from the *N*-phenoxypropyl derivative to the isosteric phenoxybutyl decreases the analgesic potency by a factor of ten, whereas the omission of the ether oxygen yields a slightly more potent compound [17]. A list of seven other examples is given by Schatz in the second edition of Burger's *Medicinal Chemistry* [215].

The explanation for this anomalous behavior may be that the omission of the ether oxygen yields a closer compound in terms of lipophilicity than its replacement by a methylene. An example that can be compared to Friedman's paradox is found in the resemblance of the phenylethyl type β -blockers (e.g., dichloroisoprenaline, sotalol) to the phenoxypropanol type (e.g., practolol, acebutolol).

VI. MINOR METALLOIDS-TOXIC ISOSTERES

In this section we describe some "exotic" applications of the bioisostery concept, implying the utilization of unusual elements such as silicon, boron, selenium, arsenic, and antimony. The use of those elements as bioisosteres of carbon in existing drugs is a different approach, enabling the introduction of a new drug-like chemical space into the drug discovery and development process.

A. Carbon–Silicon Bioisosterism

Silicon is directly below carbon in the periodic table, so according to the Erlenmeyer's expansion of the isosterism concept, carbon and silicon can be considered as true isosteres. Sila-substitution (C/Si exchange) of biologically active substances is an approach to search for new drug-like candidates with improved pharmacological properties and stronger IP position. The application of this isosterism remains, however, limited. For reviews on the subject, see Fessenden and Fessenden [216], Tacke and Zilch [217,218], Ricci et al, [219], and Showell and Mills [220].

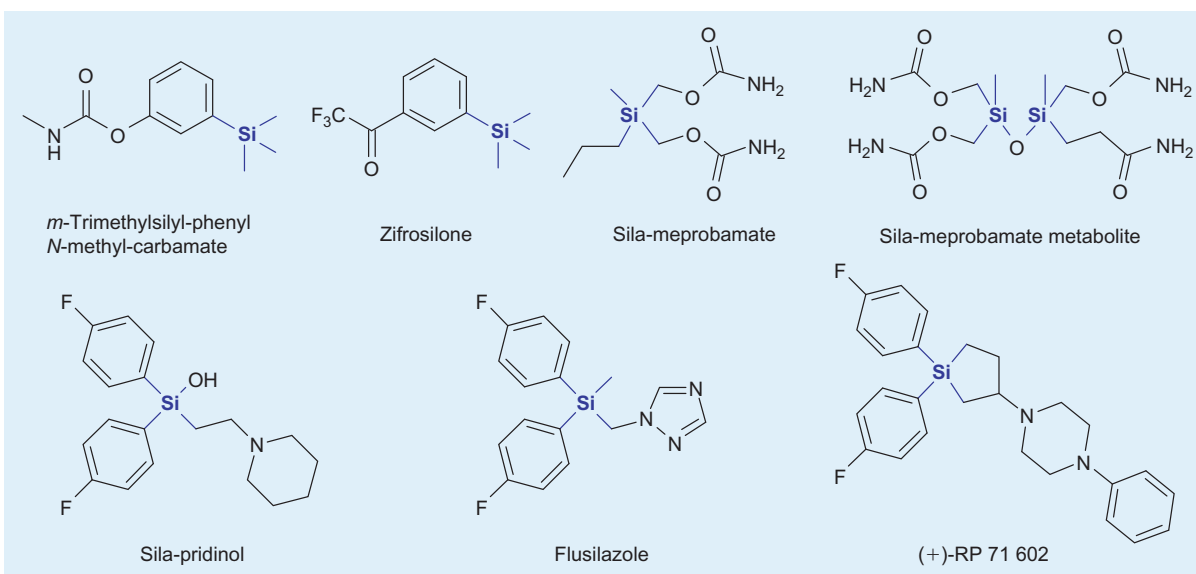


FIGURE 8.67 Organosilicon active substances.

Silicon is more electropositive than carbon (and even more if compared to oxygen and nitrogen) and the covalent silicon–carbon bonds in the sp^3 hybridization state are 20 percent longer than the corresponding carbon–carbon bond. This difference is reflected on the properties of the silaisosteres compared to the corresponding carbon analogs. For example, acidity, the hydrogen bond strength of the silanol is more favorable as a donor than that of carbinol. In this respect, the silanol isosteres may be beneficial in those pharmacophores where the H-bonding has a predominant role. From a lipophilicity point of view, the silicon-containing analogs are more lipophilic than their carbon analogs, and when an increase in lipophilicity is sought this stratagem could be used. The $C=O$ double bond of a ketone is quite stable, whereas the formation of a $Si=O$ double bond is disfavored over its hydrate form, the silicon diol. The chemistry of silicon relies mainly on the chemistry of single bonds, which have led to its appropriate use as a tetrahedral bioisostere of carbon. In Figure 8.67, some of examples of the sila-substitution of existing drugs are shown. Among these, *m*-trimethylsilyl-phenyl *N*-methylcarbamate and *m*-trimethylsilyl- α -trifluoroacetophenone (zifrosilone) are acetylcholinesterase inhibitors [221–223], sila-meprobamate is a CNS depressant [224], sila-pridinol is an anticholinergic [225], flusilazole is a fungicide for agricultural use [226], and (+)-RP 71,602 is a potent and selective 5-HT_{2A} antagonist [227].

Figure 8.68 shows a silicon-containing hypocholesterolemic squalene epoxidase inhibitor; [228,229] the silicon analog of the α_2 -adrenergic antagonist atipamezole; [230] a highly potent, stable, and CNS-penetrating silatecan; [231] some ACE inhibitors; [232] HIV protease inhibitors; [233] the (*R*)-sila-analog of the antidepressant venlafaxine; [234] and a trimethylsilylpyrazole as novel inhibitor of p38 MAP kinase [235] (mitogen-activating protein). As for the preceding molecules, the silicon atom of these compounds is quaternary and thus expected to be less sensitive to metabolic degradation (Figure 8.68).

Compared to their carbon bioisosteres, silicon-containing molecules are more sensitive to hydrolysis and to nucleophilic attack in general. Even when located in the center of a quaternary structure, the risk exists that the silicon atom will be attacked. Thus, 1-chloro-1-sila-bicyclo-(2,2,1)-heptane can still be hydrolyzed by an attack on the vacant d orbital [236]. This attack is lateral and therefore possible even in cases where the corresponding carbon derivative would have been inert toward a S_N2 reaction (Figure 8.69).

This sensitivity toward lateral attacks explains the four times shorter duration of action of sila-meprobamate compared to its carbon isostere on a model of tranquillizing activity in mice (rotarod test, potentiation of hexobarbital-induced sleep, and intraperitoneal injection) [224]. On the other hand, when given orally, sila-meprobamate is practically inactive. One of the first metabolites formed has been characterized as being a di-siloxane [216] (Figure 8.67). For the two phenyl-trimethylsilyl-derived AChE inhibitors, the rather positively charged trimethyl-silyl group mimics the trimethyl-ammonium function present in

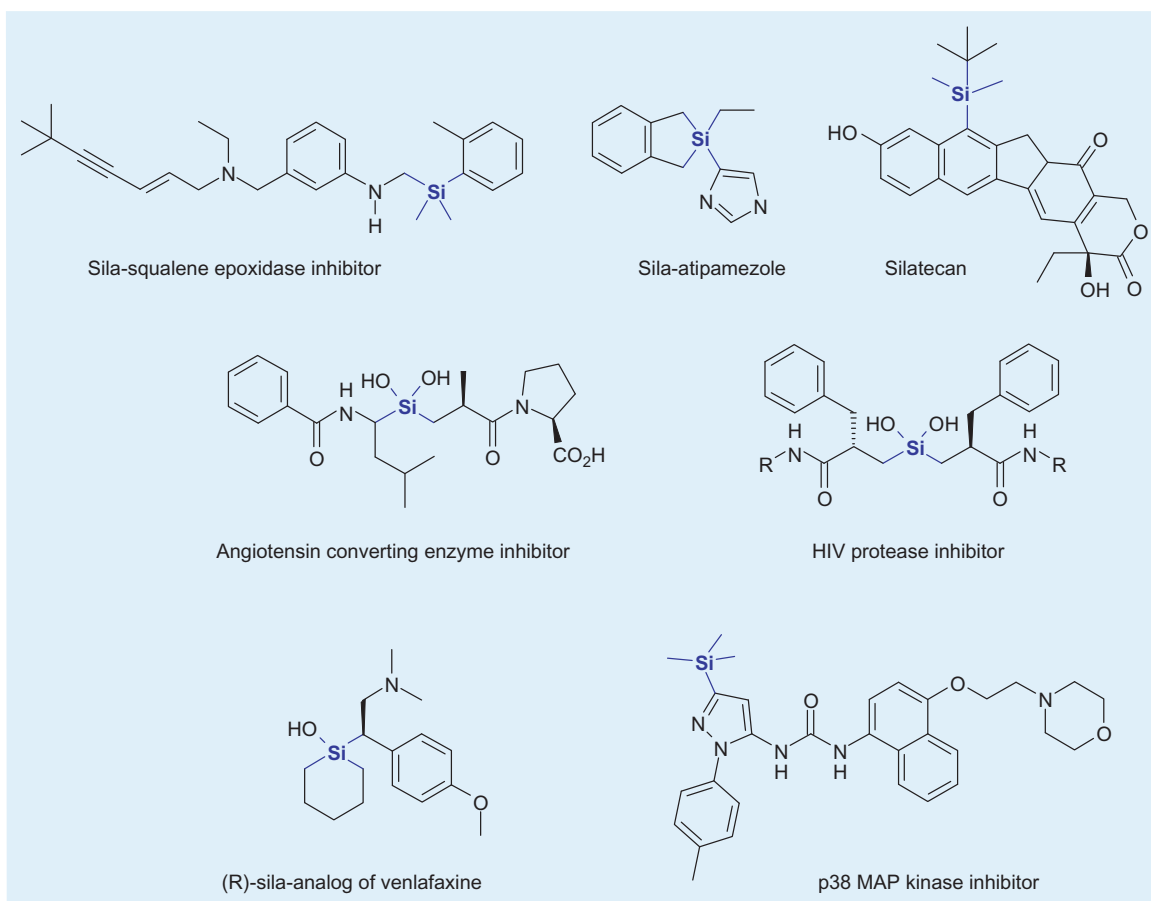


FIGURE 8.68 Structures of silicon-containing compounds which are biologically active.

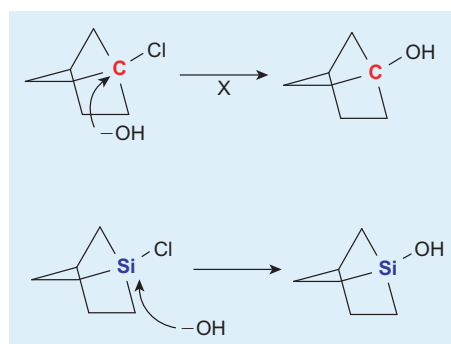


FIGURE 8.69 Due to the presence of a vacant d orbital, a lateral attack can substitute for dorsal attacks in organo-silicon derivatives [236].

acetylcholine. For these compounds, metabolic oxidation does not take place on the silicon but on one of methyl groups ($\text{Si-CH}_3 \rightarrow \text{Si-CH}_2\text{-OH}$) [221].

B. Carbon–Boron Isosterism

Boron is essential for plant growth and development. In medicinal chemistry, its main use is related to that of coupling reagent. The most important employment of boron as a drug is in the treatment of certain tumors by

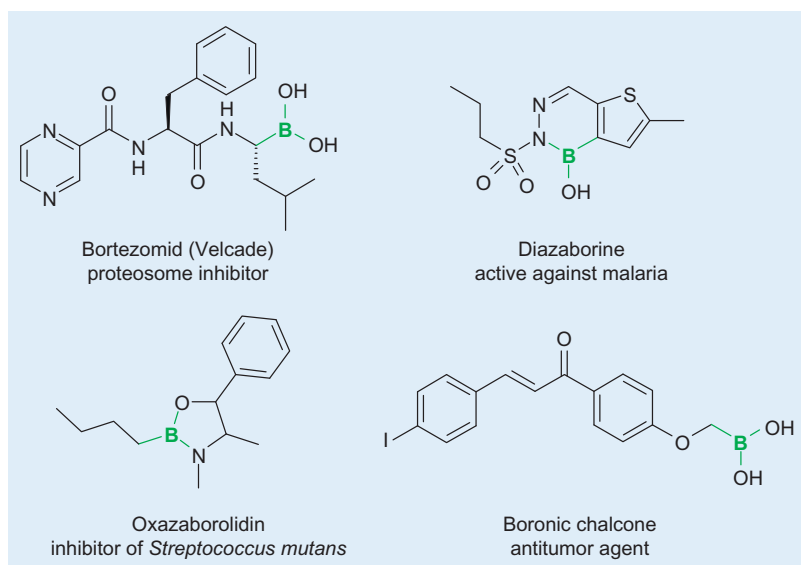


FIGURE 8.70 Boron-containing molecules with a biological activity.

Boron Neutron Capture Therapy (BNCT) [237–239]. BNCT is a binary treatment modality that can selectively irradiate tumor tissue. The delivery to the tumor cells of a drug containing the ^{10}B isotope followed by a low energy irradiation (neutrons) causes the ^{10}B to split, releasing an alpha particle a lithium nucleus. These products of the $^{10}\text{B}(\text{n},\alpha)^7\text{Li}$ reaction are very damaging to cells but have a combined path length in tissue of approximately 14 μm , or roughly the diameter of two cells. Thus, most of the ionizing energy imparted to tissue is localized to 10B-loaded cells. The problem here is to ensure a sufficient concentration of the product in the tumor being treated.

Few medicines based on boron are known. In general, boric acid or a boronic acid serve to esterify an α -diol or an ortho-diphenol. This is the case for the emetic antimony borotartrates of the ancient pharmacopoeias; for the injectable catecholamine solutions; for tolboxane [240], which is close to meprobamate and was commercially available as a tranquillizer some decades ago; and for the phenylboronic esters of chloramphenicol [241]. Boromycine was the first natural product containing boron isolated [242]. It is a complex between boric acid and a polyhydroxylated tetradentate macrocycle [243]. Another natural product is aplasmomycin with antibiotic properties [244].

Some of the boron-containing molecules biologically active are reported in Figure 8.70. Some boronic analogs of amino acids were prepared as chymotrypsin and elastase inhibitors [245], and more recently as an antineoplastic agent (i.e., Velcade, a proteasome inhibitor reported in Figure 8.70) [246]. Carboxyboranes complexed with a tertiary amine ($\text{R}_3\text{N} \cdot \text{H}_2\text{B} \cdot \text{COOH}$) are considered boron amino acid isosteres due to the isoelectronic features. Compounds-containing carboxy boranes have shown anticancer, hypolipidemic, and antifungal activity [247]. Diazaborines are active against malaria [248], and oxazaborolidines possess antibacterial activity (Figure 8.70) [249]. Boronic chalcones are reported to be antitumor agents (Figure 8.70) [250].

Organoboron derivatives—even more than organosilicon compounds—are sensitive to hydrolytic degradation, which always leads to the final formation of boric acid. But boric acid has teratogenic properties in chickens. It produces the same malformations as those produced by a riboflavin (vitamin B_2) deficiency, and the administration of riboflavin prevents these toxic effects [251,252]. The mechanism by which boric acid produces a deficiency in riboflavin is not known. In humans, the chronic utilization of boron derivatives results in cases of borism (dry skin, cutaneous eruptions, and gastric troubles) [253].

C. Bioisosteres Involving Selenium

Selenium can be considered the best isoster of sulfur, as it is just below it in the periodic table. These two atoms have very similar physical properties: the radius of selenium is only 12.5 percent bigger than that of sulfur, and their electronegativity is rather similar.

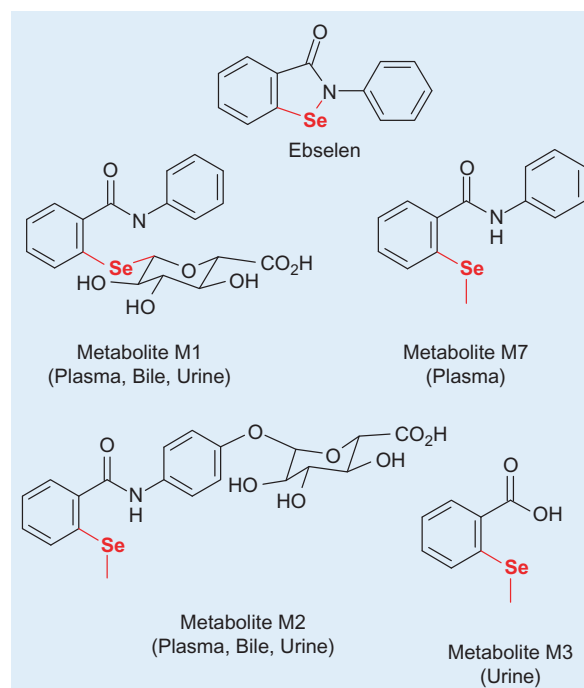


FIGURE 8.71 Ebselen and its main metabolites [256].

Selenium and its derivatives are highly toxic and, with the exception of ^{75}Se derivatives that serve diagnostic purposes (e.g., ^{75}Se -selenomethionine, used as a radioactive imaging agent in pancreatic scanning), there is no chemically defined seleno-organic drug on the market. Klayman reviewed a large number of selenium derivatives as chemotherapeutic agents in 1973 [254]. Selenium bioisosteres of sulfur compounds are mainly used as research tools (e.g., bis [2-chloroethyl] selenide as selenium bioisostere of the classical sulfur mustards [255]). Selenocysteine is present in the catalytic site of mammalian glutathione-peroxidase, and this explains the importance of selenium as an essential trace element.

The only selenium-containing drug candidate is *ebselen* (Figure 8.71) which owes its antioxidant and anti-inflammatory properties to its interference with the selenoenzyme glutathione-peroxidase [257]. Due to its strongly bound selenium moiety, only metabolites of low toxicity are formed [256].

References

- [1] Langmuir I. The structure of atoms and the octet theory of valence. *Proc Natl Acad Sci USA* 1919;5(7):252–9.
- [2] Friedman HL. Influence of isosteric replacements upon biological activity. *NASNRS* 1951;206:295–358.
- [3] Allen HS. Molecular frequency and molecular number. Parts I–III. London: n.p.; 1918.
- [4] Langmuir I. Isomorphism, isosterism, and covalence. *J Amer Chem Soc* 1919;41:1543–59.
- [5] Patani GA, LaVoie EJ. Bioisosterism: a rational approach in drug design. *Chem Rev* 1996;96(8):3147–76.
- [6] Seifriz W. Pathogenicity and isosterism. *Science* 1948;107:15–16.
- [7] Grimm HG. Structure and size of the non-metallic hybrids. *Z Electrochem* 1925;31:474–80.
- [8] Grimm HG. On the systematic arrangement of chemical compounds from the perspective of research on atomic composition; and on some challenges in experimental chemistry. *Naturwissenschaften* 1928;17:557–64.
- [9] Erlenmeyer H, Leo M. On pseudoatoms. *Helv Chim Acta* 1932;15:1171–86.
- [10] Gelmboldt VO, Ennan AA, Ganin EV, Simonov YA, Fonari MS, Botoshansky MM. Synthesis and structure of fluorosilicic acid compounds with 4-aminobenzoic acid and with 4-aminobenzenesulfonamide: the role of H-bonding in crystal structure formation. *J Fluorine Chem* 2004;125:1951–7.
- [11] McLeod JW, Mayr-Harting A, Walker N. Observations on the bactericidal and bacteriostatic actions of p-aminobenzenesulfonamide and p-hydroxylaminobenzenesulfonamide, with special reference to their suppression by p-aminobenzoic acid. *Br J Exp Pathol* 1944;25:27–37.
- [12] Brauner-Osborne H, Krogsgaard-Larsen P. Pharmacology of (S)-homoquisqualic acid and (S)-2-amino-5-phosphonopentanoic acid [(S)-AP5] at cloned metabotropic glutamate receptors. *Br J Pharmacol* 1998;123(2):269–74.

- [13] Malachowski WP, Coward JK. The chemistry of phosphopeptides: investigations on the synthesis of phosphonamide, phosphonate, and phosphinate analogues of glutamyl- γ -glutamate. *J Org Chem* 1994;59(25):7625–34.
- [14] Thornber CW. Isosterism and molecular modification in drug design. *Chem Soc Rev* 1957;8:563–80.
- [15] Burger A, editor. *Medicinal chemistry*. 3rd ed. New York: Wiley-Interscience ed; 1970.
- [16] Lima LM, Barreiro EJ. Bioisosterism: a useful strategy for molecular modification and drug design. *Curr Med Chem* 2005;12(1):23–49.
- [17] Janssen PAJ, Van der Eycken CAM. The chemical anatomy of potent morphine-like analgesics (p. 42). In: Burger A, editor. *Drugs affecting the central nervous system*. New York: Marcel Dekker, Inc.; 1968. p. 25–60.
- [18] Boyle EA, Mangan FR, Markwell RE, Smith SA, Thomson MJ, Ward RW, et al. 7-Aroyl-2,3-dihydrobenzo[b]furan-3-carboxylic acids and 7-benzoyl-2,3-dihydrobenzo[b]thiophene-3-carboxylic acids as analgesic agents. *J Med Chem* 1986;29:894–8.
- [19] Morgan BP, Scholtz JM, Ballinger MD, Zipkin ID, Bartlett PA. Differential binding energy: a detailed evaluation of the influence of hydrogen bonding and hydrophobic groups on the inhibition of thermolysin by phosphorous-containing inhibitors. *J Am Chem Soc* 1991;113:297–307.
- [20] Chen YL, Nielsen J, Hedberg KD, Dunaikis A, Jones S, Russo L, et al. Syntheses, resolution, and structure-activity relationships of potent acetylcholinesterase inhibitors: 898-carbaphysostigmine analogues. *J Med Chem* 1992;35:1429–34.
- [21] Marseigne I, Roques BP. Synthesis of new amino-acids mimicking sulfated and phosphorylated tyrosine residues. *J Org Chem* 1988;53:3621–4.
- [22] Burke Jr TR, Smyth M, Nomizu M, Otake A, Roller PP. Preparation of fluoro- and hydroxy-4-phosphonomethyl-d,l-phenylalanine suitably protected for solid phase synthesis of peptides containing hydrolytically stable analogues of O-phosphotyrosine. *J Org Chem* 1993;58:1336–40.
- [23] Erlenmeyer H, Willi E. Zusammenhänge zwischen Konstitution und Wirkung bei Pyrazolonderivaten [Relationships between structure and effect in pyrazolone derivatives]. *Helv Chim Acta* 1935;18:740–3 [in German].
- [24] Wallace EM, Moliterni JA, Moskal MA, Neubert AD, Marcopoulos N, Stamford LB, et al. Design and synthesis of potent selective inhibitors of endothelin-converting enzyme. *J Med Chem* 1998;41:1513–23.
- [25] De Lombaert S, Stamford LB, Blanchard L, Tan J, Hoyer D, Diefenbacher CG, et al. Potent non-peptidic dual inhibitors of endothelin-converting enzyme and neutral endopeptidase 24.11. *Bioorg Med Chem Lett* 1997;7:1059–64.
- [26] Binder D, Noe CR, Holzer W, Baumann K. Thiophen als Strukturelement physiologisch aktiver Substanzen, 16. Mitt. Thienoisoxazole durch Substitution am Oximstickstoff [Thiophene as a structural element of physiologically active compounds, XVI: thienoisoxazoles by substitution at the oxime nitrogen]. *Arch Pharm* 1987;320:837–43 [in German].
- [27] Uno H, Kurokawa M, Masuda Y, Nishimura H. Studies on 3-substituted 1,2-benzisoxazole derivatives. 6. Syntheses of 3-(sulfamoylmethyl)-1,2-benzisoxazole derivatives and their anticonvulsant activities. *J Med Chem* 1979;22:180–3.
- [28] Garvey DS, Wasicak JT, Decker MW, Brioni JD, Buckley MJ, Sullivan JP, et al. Novel isoxazoles which interact with brain cholinergic channel receptors have intrinsic cognitive enhancing and anxiolytic activities. *J Med Chem* 1994;37(8):1055–9.
- [29] Garvey DS, Wasicak JT, Elliott RL, Lebold SA, Hettinger AM, Carrera GM, et al. Ligands for brain cholinergic channel receptors: synthesis and in vitro characterization of novel isoxazoles and isothiazoles as bioisosteric replacements for the pyridine ring in nicotine. *J Med Chem* 1994;37(26):4455–63.
- [30] Olesen PH, Tonder JE, Hansen JB, Hansen HC, Rimvall K. Bioisosteric replacement strategy for the synthesis of 1-azacyclic compounds with high affinity for the central nicotinic cholinergic receptors. *Bioorg Med Chem* 2000;8(6):1443–50.
- [31] Gohlke H, Gundisch D, Schwarz S, Seitz G, Tilotta MC, Wegge T. Synthesis and nicotinic binding studies on enantiopure diazine analogues of the novel (2-chloro-5-pyridyl)-9-azabicyclo[4.2.1]non-2-ene UB-165. *J Med Chem* 2002;45(5):1064–72.
- [32] Heinisch G, Holzer W, Kunz F, Langer T, Lukavsky P, Pechlaner C, et al. On the bioisosteric potential of diazines: diazine analogues of the combined thromboxane A2 receptor antagonist and synthetase inhibitor ridogrel. *J Med Chem* 1996;39(20):4058–64.
- [33] Haginoya N, Kobayashi S, Komoriya S, Yoshino T, Suzuki M, Shimada T, et al. Synthesis and conformational analysis of a non-amidine factor Xa inhibitor that incorporates 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine as S4 binding element. *J Med Chem* 2004;47(21):5167–82.
- [34] Wood MR, Schirripa KM, Kim JJ, Wan BL, Murphy KL, Ransom RW, et al. Cyclopropylamino acid amide as a pharmacophoric replacement for 2,3-diaminopyridine. Application to the design of novel bradykinin B1 receptor antagonists. *J Med Chem* 2006;49(4):1231–4.
- [35] Abe Y, Kayakiri H, Satoh S, Inoue T, Sawada Y, Inamura N, et al. A novel class of orally active non-peptide bradykinin B2 receptor antagonists. 3. Discovering bioisosteres of the imidazo[1,2-a] pyridine moiety. *J Med Chem* 1998;41(21):4062–79.
- [36] Contreras JM, Parrot I, Sippl W, Rival YM, Wermuth CG. Design, synthesis, and structure-activity relationships of a series of 3-[2-(1-benzylpiperidin-4-yl)ethylamino]pyridazine derivatives as acetylcholinesterase inhibitors. *J Med Chem* 2001;44(17):2707–18.
- [37] Contreras JM, Rival YM, Chayer S, Bourguignon JJ, Wermuth CG. Aminopyridazines as acetylcholinesterase inhibitors. *J Med Chem* 1999;42(4):730–41.
- [38] Wermuth CG. *The practice of medicinal chemistry*. 2nd ed. London: Academic Press; 2003. p. 768.
- [39] Biava M, Porretta GC, Cappelli A, Vomero S, Manetti F, Botta M, et al. 1,5-Diarylpyrrole-3-acetic acids and esters as novel classes of potent and highly selective cyclooxygenase-2 inhibitors. *J Med Chem* 2005;48(9):3428–32.
- [40] Barbachyn MR, Ford CW. Oxazolidinone structure-activity relationships leading to linezolid. *Angew Chem Int Ed Engl* 2003;42(18):2010–23.
- [41] Renslo AR, Luehr GW, Gordeev MF. Recent developments in the identification of novel oxazolidinone antibacterial agents. *Bioorg Med Chem* 2006;14(12):4227–40.
- [42] Snyder LB, Meng Z, Mate R, D'Andrea SV, Marinier A, Quesnelle CA, et al. Discovery of isoxazolinone antibacterial agents. Nitrogen as a replacement for the stereogenic center found in oxazolidinone antibacterials. *Bioorg Med Chem Lett* 2004;14(18):4735–9.
- [43] Niluis AM. Have the oxazolidinones lived up to their billing? Future perspectives for this antibacterial class. *Curr Opin Investig Drugs* 2003;4(2):149–55.
- [44] Ganellin CR. Discovery of cimetidine, ranitidine and other H₂-receptor histamine antagonists. In: Ganellin CR, Roberts SM, editors. *Medicinal chemistry: The role of organic chemistry in drug research*. London: Academic Press; 1993. p. 227–55.

II. LEAD COMPOUND DISCOVERY STRATEGIES

- [45] Mallamo JP, Pilling GM, Wetzel JR, Kowalczyk PJ, Bell MR, Kullnig RK, et al. Antiandrogenic steroidal sulfonyl heterocycles. Utility of electrostatic complementarity in defining bioisosteric sulfonyl heterocycles. *J Med Chem* 1992;35:1663–70.
- [46] Fludzinski P, Evrard DA, Bloomquist WE, Lacefield WB. Indazoles as indole bioisosteres: synthesis and evaluation of the tropanyl ester and amide of indazole-3-carboxylate as antagonists to the serotonin 5HT₃ receptor. *J Med Chem* 1987;30:1535–7.
- [47] Mathes BM, Hudziak KJ, Schaus JM, Xu YC, Nelson DL, Wainscott DB, et al. Substituted furo[3,2-b]pyridines: novel bioisosteres of 5-HT 1F receptor agonists. *Bioorg Med Chem Lett* 2004;14(1):167–70.
- [48] Blair JB, Marona-Lewicka D, Kanthasamy A, Lucaites VL, Nelson DL, Nichols DE. Thieno[3,2-b]- and thieno[2,3-b]pyrrole bioisosteric analogues of the hallucinogen and serotonin agonist N, N-dimethyltryptamine. *J Med Chem* 1999;42(6):1106–11.
- [49] Yous S, Andrieux J, Howell HE, Morgan PJ, Renard P, Pfeiffer B, et al. Novel naphthalenic ligands with high affinity for the melatonin receptor. *J Med Chem* 1992;35(8):1484–6.
- [50] Depreux P, Lesieur D, Mansour HA, Morgan P, Howell HE, Renard P, et al. Synthesis and structure-activity relationships of novel naphthalenic and bioisosteric related amidic derivatives as melatonin receptor ligands. *J Med Chem* 1994;37(20):3231–9.
- [51] Blaskó G, Major E, Blaskó G, Rózsa I, Szántay C. Pyrimido[1,6-a]pyrido[3,4-b] indoles as new platelet inhibiting agents. *Eur J Med Chem* 1986;21:91–5.
- [52] Kardos J, Blaskó G, Simonyi M, Szántay C. Octahydroindolo[2,3-a]quinolizin-2-one, a novel structure for γ -aminobutyric acid (GABA) uptake inhibition. *Eur J Med Chem* 1985;21:151–4.
- [53] Salituro FG, Harrison BL, Baron BM, Nyce PL, Stewart KT, Kehne JH, et al. 3-(2-Carboxyindol-3-yl)propionic acid-based antagonists of the N-methyl-D-aspartic receptor associated glycine binding site. *J Med Chem* 1992;35:1791–9.
- [54] Calvino R, Stilo A, Di Fruttero R, Gasco AM, Sorba G, Gasco A. Pharmacochimistry of the furoxan ring: recent developments. *Il Farmaco* 1993;48:321–34.
- [55] Lipinski CA, Aldinger CE, Beyer TA, Bordner J, Burdi DF, Bussolotti DL, et al. Hydantoin isosteres: in vivo active spiro hydroxy acetic aldose reductase inhibitors. *J Med Chem* 1992;35:2169–77.
- [56] Lober S, Hubner H, Gmeiner P. Fused azaindole derivatives: molecular design, synthesis and in vitro pharmacology leading to the preferential dopamine D3 receptor agonist FAUC 725. *Bioorg Med Chem Lett* 2002;12(17):2377–80.
- [57] Krosgaard-Larsen P, Hjedts H, Falch E, Jørgensen FS, Nielsen L. Recent advances in GABA agonists, antagonists and uptake inhibitors: structure-activity relationships and therapeutic potential. In: Testa B, editor. *Advances in drug research*, vol. 17. London: Academic Press; 1988. p. 381–456.
- [58] Almquist RG, Chao WR, Jennings-White C. Synthesis and biological activity of carboxylic acid replacement analogues of the potent angiotensin converting enzyme inhibitor 5(S)-benzamido-4-oxo-6-phenylhexanoyl-L-proline. *J Med Chem* 1985;28:1067–71.
- [59] Massa S, Mai A, Sbardella G, Esposito M, Ragno R, Loidl P, et al. 3-(4-Aroyl-1H-pyrrol-2-yl)-N-hydroxy-2-propenamides, a new class of synthetic histone deacetylase inhibitors. *J Med Chem* 2001;44(13):2069–72.
- [60] Lu Q, Yang YT, Chen CS, Davis M, Byrd JC, Etherton MR, et al. Zn²⁺-chelating motif-tethered short-chain fatty acids as a novel class of histone deacetylase inhibitors. *J Med Chem* 2004;47(2):467–74.
- [61] Remiszewski SW, Sambucetti LC, Bair KW, Bontempo J, Cesarz D, Chandramouli N, et al. N-hydroxy-3-phenyl-2-propenamides as novel inhibitors of human histone deacetylase with in vivo antitumor activity: discovery of (2E)-N-hydroxy-3-[4-[(2-hydroxyethyl)[2-(1H-indol-3-yl)ethyl]amino]methyl]phenyl]-2-propenamide (NVP-LAQ824). *J Med Chem* 2003;46(21):4609–24.
- [62] Plumb JA, Finn PW, Williams RJ, Bandara MJ, Romero MR, Watkins CJ, et al. Pharmacodynamic response and inhibition of growth of human tumor xenografts by the novel histone deacetylase inhibitor PXD101. *Mol Cancer Ther* 2003;2(8):721–8.
- [63] Kelly WK, O'Connor OA, Krug LM, Chiao JH, Heaney M, Curley T, et al. Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. *J Clin Oncol* 2005;23(17):3923–31.
- [64] Buggy JJ, Cao ZA, Bass KE, Verner E, Balasubramanian S, Liu L, et al. CRA-024781: a novel synthetic inhibitor of histone deacetylase enzymes with antitumor activity in vitro and in vivo. *Mol Cancer Ther* 2006;5(5):1309–17.
- [65] Hanessian S, Moitessier N, Gauchet C, Viau M. N-aryl sulfonyl homocysteine hydroxamate inhibitors of matrix metalloproteinases: further probing of the S(1), S(1)9, and S(2)9 pockets. *J Med Chem* 2001;44(19):3066–73.
- [66] Aranapakam V, Davis JM, Grosu GT, Baker J, Ellingboe J, Zask A, et al. Synthesis and structure-activity relationship of N-substituted 4-arylsulfonylpiperidine-4-hydroxamic acids as novel, orally active matrix metalloproteinase inhibitors for the treatment of osteoarthritis. *J Med Chem* 2003;46(12):2376–96.
- [67] Aranapakam V, Grosu GT, Davis JM, Hu B, Ellingboe J, Baker JL, et al. Synthesis and structure-activity relationship of alpha-sulfonylhydroxamic acids as novel, orally active matrix metalloproteinase inhibitors for the treatment of osteoarthritis. *J Med Chem* 2003;46(12):2361–75.
- [68] Noe MC, Natarajan V, Snow SL, Mitchell PG, Lopresti-Morrow L, Reeves LM, et al. Discovery of 3,3-dimethyl-5-hydroxy-pipecolic hydroxamate-based inhibitors of aggrecanase and MMP-13. *Bioorg Med Chem Lett* 2005;15(11):2808–11.
- [69] Duan JJ, Chen L, Wasserman ZR, Lu Z, Liu RQ, Covington MB, et al. Discovery of gamma-lactam hydroxamic acids as selective inhibitors of tumor necrosis factor alpha converting enzyme: design, synthesis, and structure-activity relationships. *J Med Chem* 2002;45(23):4954–7.
- [70] Kohler HV, Eichler B, Salewski R. Untersuchungen zum sauerstoffanalogen Charakter der C(CN)₂- und NCN- Gruppen [Studies in the oxygen analog character of the C(CN)₂- and NCN- groups]. *Z Anorg Chem* 1970;379:183–92 [in German].
- [71] Kwon C-H, Nagasawa HT, DeMaster EG, Shiota FN. Acyl, N-protected α -aminoacyl, and peptidyl derivatives as prodrug forms of the alcohol deterrent agent cyanamide. *J Med Chem* 1986;29:1922–9.
- [72] Drummond JT, Johnson G. Convenient procedure for the preparation of alkyl end aryl substituted N-(aminoalkylacyl)sulfonamides. *Tetrahedron Lett* 1988;29:1653–6.
- [73] Albright JD, DeVries VG, Du MD, Largis EE, Miner TG, Reich MF, et al. Potential antiatherosclerotic agents. 3. Substituted benzoic and non benzoic analogues of cetabon. *J Med Chem* 1983;26:1393–411.
- [74] Uehling DE, Donaldson KH, Deaton DN, Hyman CE, Sugg EE, Barrett DG, et al. Synthesis and evaluation of potent and selective beta(3) adrenergic receptor agonists containing acylsulfonamide, sulfonylsulfonamide, and sulfonylurea carboxylic acid isosteres. *J Med Chem* 2002;45(3):567–83.

II. LEAD COMPOUND DISCOVERY STRATEGIES

- [75] Johansson A, Poliakov A, Akerblom E, Wiklund K, Lindeberg G, Winiwarter S, et al. Acyl sulfonamides as potent protease inhibitors of the hepatitis C virus full-length NS3 (protease-helicase/NTase): a comparative study of different C-terminals. *Bioorg Med Chem* 2003;11(12):2551–68.
- [76] Bovy PR, Reitz DB, Collins JT, Chamberlain TS, Olins GM, Corpus VM, et al. Nonpeptide angiotensin II antagonists: N-phenyl-1-H-pyrrole derivatives are angiotensin II receptor antagonists. *J Med Chem* 1993;36:101–10.
- [77] Marshall WS, Goodson T, Cullinan GJ, Swanson-Bean D, Haisch KD, Rinkema LE, et al. Leukotriene receptor antagonists. 1. Synthesis and structure-activity relationships of alkoxyacetophenone derivatives. *J Med Chem* 1987;30:682–9.
- [78] Krogsgaard-Larsen P. In: Hansch C, Sammes PG, Taylor JB, Emmet JC, editors. *Comprehensive medicinal chemistry*, vol. 3. Oxford: Pergamon Press; 1990. p. 493–537.
- [79] Lunn WHW, Schoepp DD, Lodge D, True RA, Millar JD. Poster N P-041.A. LY262466, DL-2-amino-3-(4-hydroxy-1,2,5-thiazol-3-yl) propanoic acid hydrochloride, a novel and selective agonist at the AMPA excitatory amino acid receptor. 12th International Symposium on Medicinal Chemistry 1992;pp. 113–17.
- [80] Atkinson JG, Girard Y, Rokach J, Rooney CS, McFarlane CS, Rackham A, et al. Kojic amine-A novel γ -aminobutyric acid analogue. *J Med Chem* 1979;22:90–106.
- [81] Hulin B, McCarthy PA, Gibbs EM. The glitazone family of antidiabetic agents. *Curr Pharm Des* 1996;2:85–102.
- [82] Henke BR. Peroxisome proliferator-activated receptor alpha/gamma dual agonists for the treatment of type 2 diabetes. *J Med Chem* 2004;47(17):4118–27.
- [83] Gezginci MH, Martin AR, Franzblau SG. Antimycobacterial activity of substituted isosteres of pyridine- and pyrazinecarboxylic acids. 2. *J Med Chem* 2001;44(10):1560–3.
- [84] Nicolaou I, Zika C, Demopoulos VJ. [1-(3,5-Difluoro-4-hydroxyphenyl)-1H-pyrrol-3-yl]phenylmethanone as a bioisostere of a carboxylic acid aldose reductase inhibitor. *J Med Chem* 2004;47(10):2706–9.
- [85] Qiu J, Stevenson SH, O'Beirne MJ, Silverman RB. 2,6-Difluorophenol as a bioisostere of a carboxylic acid: bioisosteric analogues of gamma-aminobutyric acid. *J Med Chem* 1999;42(2):329–32.
- [86] Noe MC, Natarajan V, Snow SL, Wolf-Gouveia LA, Mitchell PG, Lopresti-Morrow L, et al. Discovery of 3-OH-3-methylpipercolic hydroxamates: potent orally active inhibitors of aggrecanase and MMP-13. *Bioorg Med Chem Lett* 2005;15(14):3385–8.
- [87] Buu-Hoi NP, Lambelin G, Lepoivre C, Gillet C, Gautier M, Thiriaux J. Un nouvel agent anti-inflammatoire de structure non stéroïdique: l'acide p-butoxyphénylacéthydroxamique [A new anti-inflammatory agent of non-steroidal structure: P-butoxyphenylacethydroxamic acid]. *C R Acad Sci (Paris)* 1965;261:2259–62.
- [88] Orzalesi G, Sellaeri R. Pharmaceutical 2-(4-isobutylphenyl) propionohydroxamic acid. Ger. Offen. 2,400,531 (24 Jul. 1974, to Societa Italo-Britannica L. Manetti & H.Roberts e C.). 1974;C.A. 1974; 81; 120272i.
- [89] De Martis F, Franzone JS, Tamietto T. Sintesi e proprietà antiflogistiche di alcuni acidi indolil-acetoidrossammici [Synthesis and anti-inflammatory properties of various indolyl-acetohydroxamic acids]. *Bol Chim Farm* 1975;114:309–18 [in Italian].
- [90] Orzalesi G, Mari F, Bertol E, Sellaeri R, Pisaturo G. Anti-inflammatory agents: determination of ibuprofen and its metabolite in humans. *Arzneim-Forsch* 1980;30:1607–9.
- [91] Demay F, De Sy J. A new non-steroidal anti-inflammatory drug (NSAID) in current rheumatologic practice (oxametacin). *Curr Ther Res* 1982;31:113–18.
- [92] Vergin H, Ferber H, Brunner F, Kukovetz WR. Pharmakokinetik und Biotransformation von Oxametacin bei gesunden Probanden [Pharmacokinetics and biotransformation of oxametacin in healthy volunteers]. *Arzneim Forsch* 1981;31:513–18 [in French].
- [93] Allegretti M, Bertini R, Cesta MC, Bizzarri C, Di Bitondo R, Di Cioccio V, et al. 2-Arylpropionic CXC chemokine receptor 1 (CXCR1) ligands as novel noncompetitive CXCL8 inhibitors. *J Med Chem* 2005;48(13):4312–31.
- [94] Wan Y, Wallinder C, Johansson B, Holm M, Mahalingam AK, Wu X, et al. First reported nonpeptide AT1 receptor agonist (L-162,313) acts as an AT2 receptor agonist in vivo. *J Med Chem* 2004;47(6):1536–46.
- [95] Lobb KL, Hipskind PA, Aikins JA, Alvarez E, Cheung YY, Considine EL, et al. Acyl sulfonamide anti-proliferatives: benzene substituent structure-activity relationships for a novel class of antitumor agents. *J Med Chem* 2004;47(22):5367–80.
- [96] Mader MM, Shih C, Considine E, Dios AD, Grossman CS, Hipskind PA, et al. Acyl sulfonamide anti-proliferatives. Part 2: activity of heterocyclic sulfonamide derivatives. *Bioorg Med Chem Lett* 2005;15(3):617–20.
- [97] Hattori K, Tanaka A, Fujii N, Takasugi H, Tenda Y, Tomita M, et al. Discovery of diphenyloxazole and Ndelta-Z-ornithine derivatives as highly potent and selective human prostaglandin EP(4) receptor antagonists. *J Med Chem* 2005;48(9):3103–6.
- [98] Liljebris C, Larsen SD, Ogg D, Palazuk BJ, Bleasdale JE. Investigation of potential bioisosteric replacements for the carboxyl groups of peptidomimetic inhibitors of protein tyrosine phosphatase 1B: identification of a tetrazole-containing inhibitor with cellular activity. *J Med Chem* 2002;45(9):1785–98.
- [99] Momose Y, Maekawa T, Odaka H, Ikeda H, Sohda T. Novel 5-substituted-1H-tetrazole derivatives as potent glucose and lipid lowering agents. *Chem Pharm Bull (Tokyo)* 2002;50(1):100–11.
- [100] Biot C, Bauer H, Schirmer RH, Davioud-Charvet E. 5-Substituted tetrazoles as bioisosteres of carboxylic acids: bioisosterism and mechanistic studies on glutathione reductase inhibitors as antimalarials. *J Med Chem* 2004;47(24):5972–83.
- [101] Kimura T, Shuto D, Hamada Y, Igawa N, Kasai S, Liu P, et al. Design and synthesis of highly active Alzheimer's beta-secretase (BACE1) inhibitors, KMI-420 and KMI-429, with enhanced chemical stability. *Bioorg Med Chem Lett* 2005;15(1):211–15.
- [102] Roppe J, Smith ND, Huang D, Tehrani L, Wang B, Anderson J, et al. Discovery of novel heteroarylazoles that are metabotropic glutamate subtype 5 receptor antagonists with anxiolytic activity. *J Med Chem* 2004;47(19):4645–8.
- [103] Kozikowski AP, Zhang J, Nan F, Petukhov PA, Grajkowska E, Wroblewski JT, et al. Synthesis of urea-based inhibitors as active site probes of glutamate carboxypeptidase II: efficacy as analgesic agents. *J Med Chem* 2004;47(7):1729–38.
- [104] Valgeirsson J, Nielsen EO, Peters D, Mathiesen C, Kristensen AS, Madsen U. Bioisosteric modifications of 2-arylureidobenzoic acids: selective noncompetitive antagonists for the homomeric kainate receptor subtype GluR5. *J Med Chem* 2004;47(27):6948–57.
- [105] Kraus JL. Isosterism and molecular modification in drug design: tetrazole analogue of GABA: effects on enzymes of the gamma-aminobutyrate system. *Pharmacol Res Commun* 1983;15:183–9.

II. LEAD COMPOUND DISCOVERY STRATEGIES

- [106] Hallinan EA, Tsymbalov S, Dorn CR, Pitzele BS, Hansen Jr DW, Moore WM, et al. Synthesis and biological characterization of L-N(6)-(1-iminoethyl)lysine 5-tetrazole-amide, a prodrug of a selective iNOS inhibitor. *J Med Chem* 2002;45(8):1686–9.
- [107] Schlewer G, Wermuth CG, Chambon J-P. Analogues tétrazoliques d'agents GABA-mimétiques [Tetrazole analogues of GABA-mimetics]. *Eur J Med Chem* 1984;19:181–6 [in French].
- [108] Krogsgaard-Larsen P, Ferkany JW, Nielsen EO, Madsen U, Ebert B, Johansen JS, et al. Novel class of amino acid antagonists at non-N-methyl-D-aspartic acid excitatory amino acid receptors. Synthesis, in vitro and in vivo pharmacology, and neuroprotection. *J Med Chem* 1991;34:123–30.
- [109] Krogsgaard-Larsen P, Rodolfskov-Christiansen T. GABA agonists: synthesis and structure-activity studies on analogues of isoguvacine and THIP. *Eur J Med Chem* 1979;14:157–64.
- [110] Kraus JL. Isosterism and molecular modification in drug design: new n-dipropylacetate analogs as inhibitors of succinic semi aldehyde dehydrogenase. *Pharmacol Res Commun* 1983;15:119–29.
- [111] Lichtenthaler FW, Heidel P. Intermediates in the formation of γ -pyrones from hexose derivatives: a simple synthesis of kojic acid and hydroxymaltol. *Angew Chem Int Ed* 1969;8:978–9.
- [112] Froestl W, Furet P, Hall RG, Mickel SJ, Strub D, Sprecher GV, et al. GABA_B antagonists: novel CNS-active compounds. In: Testa B, Kyburz E, Fuhrer W, Giger R, editors. *Perspectives in medicinal chemistry*. Weinheim: VHC; 1993. p. 259–72.
- [113] Rosowski A, Forsch RA, Freisheim JH, Moran RG, Wick M. Methotrexate analogues. 19. Replacement of the glutamate side chain in classical antifolates by L-homocysteic acid and L-cysteic acid: effect on enzyme inhibition and antitumor activity. *J Med Chem* 1984;27:600–4.
- [114] Watkins JC, Krogsgaard-Larsen P, Honoré T. Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. *Trends Pharm Sci* 1990;11:25–33.
- [115] Drysdale MJ, Pritchard MC, Horwell DC. Rationally designed “dipeptoid” analogues of CCK. Acid mimics of the potent and selective non peptide CCK-B receptor antagonist CI-988. *J Med Chem* 1992;35:2573–81.
- [116] Franz RD. Comparisons of pK_a and log P values of some carboxylic and phosphonic acids: synthesis and measurement. *AAPS PharmSci* 2001;3(2):E10.
- [117] Kinney WA, Lee NE, Garrison DT, Podlesny Jr EJ, Simmonds JT, et al. Bioisosteric replacement of the α -amino carboxylic functionality in 2-amino-5-phosphonopentanoic acid yields unique 3,4-diamino-3x-cyclobutene-1,2-dione containing NMDA antagonists. *J Med Chem* 1992;35:4720–6.
- [118] Gao Y, Luo J, Yao Z-J, Guo R, Zou H, Kelley J, et al. Inhibition of Grb2 SH2 domain binding by non-phosphate-containing ligands. 2. 4-(2-Malonyl)phenylalanine as a potent phosphotyrosyl mimetic. *J Med Chem* 2000;43:911–20.
- [119] Shapiro G, Floersheim P, Boelsterli J, Amstutz R, Bolliger G, Gammethaler H, et al. Muscarinic activity of the thiolactone, lactam, lactol, and thiolactol analogues of pilocarpine and a hypothetical model for the binding of agonists to the m₁ receptor. *J Med Chem* 1992;35:15–27.
- [120] Thompkins L, Lee KH. Comparison of analgesic effects of isosteric variations of salicylic acid and aspirin (acetylsalicylic acid). *J Pharm Sci* 1975;64:760–3.
- [121] Roth GJ, Stanford N, Majerus PW. Acetylation of prostaglandine synthase by aspirin. *Proc Nat Acad Sci USA* 1975;72:3073–6.
- [122] Bundgaard H. *Design of prodrugs*. Amsterdam: Elsevier; 1985. pp. 55–61.
- [123] Sauerberg P, Chen J, WoldeMussie E, Rapoport H. Cyclic carbamate analogues of pilocarpine. *J Med Chem* 1989;32:1322–6.
- [124] Sauerberg P, Kindtler JW, Nielsen L, Sheardown MJ, Honoré T. Muscarinic cholinergic agonists and antagonists of the 3-(3-alkyl-1,2,4-oxadiazol-5-yl)1,2,5,6-tetrahydropyridine type synthesis and structure-activity relationships. *J Med Chem* 1991;34:687–92.
- [125] Sauerberg P, Olesen PH, Nielsen S, Treppendahl S, Sheardown MJ, Honoré T, et al. Novel functional M₁selective muscarinic agonists. Synthesis and structure-activity relationships of 3-(1,2,5-thiadiazolyl)-1,2,5,6-tetrahydro-1-methylpyridines. *J Med Chem* 1992;35:2263–74.
- [126] Sauerberg P, Larsen JJ, Falch E, Krogsgaard-Larsen P. A novel class of conformationally restricted heterocyclic muscarinic agonists. *J Med Chem* 1986;29(6):1004–9.
- [127] Saunders J, Cassidy M, Freedman SB, Harley EA, Iversen LL, Kneen C, et al. Novel quinuclidine-based ligands for the muscarinic cholinergic receptor. *J Med Chem* 1990;33:1128–38.
- [128] Street LJ, Baker R, Book T, Reeve AJ, Saunders J, Willson T, et al. Synthesis and muscarinic activity of quinuclidinyl- and (1-azanobornyl)pyrazine derivatives. *J Med Chem* 1992;35:295–305.
- [129] Wadsworth HJ, Jenkins SM, Orlek BS, Cassidy F, Clark MSG, Brown F, et al. Synthesis and muscarinic activities of quinuclidin-3-yltriazole and -tetrazole derivatives. *J Med Chem* 1992;35:1280–90.
- [130] Jenkins SM, Wadsworth HJ, Bromidge S, Orlek BS, Wyman PA, Riley GJ, et al. Substituent variation in azabicyclic triazole- and tetrazole-based muscarinic receptor ligands. *J Med Chem* 1992;35(13):2392–406.
- [131] Bromidge SM, Brown F, Cassidy F, Clark MS, Dabbs S, Hadley MS, et al. Design of [R-(Z)]-(1- α -(methoxyimino)-1-azabicyclo[2.2.2]octane-3-acetonitrile (SB 202026), a functionally selective azabicyclic muscarinic M₁ agonist incorporating the N-methoxy imido-yl nitrile group as a novel ester bioisostere. *J Med Chem* 1997;40(26):4265–80.
- [132] Toja E, Bonetti C, Butti A, Hunt P, Fortin M, Barzaghi F, et al. 1-Alkyl-1,2,5,6-tetrahydropyridine-3-carboxaldehyde-O-alkyl-oximes: a new class of potent orally available active muscarinic agonists related to arecoline. *Eur J Med Chem* 1991;22:853–68.
- [133] Carroll FI. 2002 Medicinal chemistry division award address: monoamine transporters and opioid receptors. Targets for addiction therapy. *J Med Chem* 2003;46(10):1775–94.
- [134] Petukhov PA, Zhang M, Johnson KJ, Tella SR, Kozikowski AP. Sar studies of piperidine-based analogues of cocaine. Part 3: oxadiazoles. *Bioorg Med Chem Lett* 2001;11(16):2079–83.
- [135] Street LJ, Baker R, Castro JL, Chambers MS, Guiblin AR, Hobbs SC, et al. Synthesis and serotonergic activity of 5-(oxadiazolyl)tryptamines: potent agonists for 5-HT_{1D} receptors. *J Med Chem* 1993;36(11):1529–38.
- [136] Kuduk SD, Ng C, Feng DM, Wai JM, Chang RS, Harrell CM, et al. 2,3-Diaminopyridine bradykinin B₁ receptor antagonists. *J Med Chem* 2004;47(26):6439–42.

II. LEAD COMPOUND DISCOVERY STRATEGIES

- [137] Borzilleri RM, Zheng X, Qian L, Ellis C, Cai ZW, Wautlet BS, et al. Design, synthesis, and evaluation of orally active 4-(2,4-difluoro-5-(methoxycarbamoyl)phenylamino)pyrrolo[2,1-f][1,2,4]triazines as dual vascular endothelial growth factor receptor-2 and fibroblast growth factor receptor-1 inhibitors. *J Med Chem* 2005;48(12):3991–4008.
- [138] Sakamoto T, Cullen MD, Hartman TL, Watson KM, Buckheit RW, Pannecouque C, et al. Synthesis and anti-HIV activity of new metabolically stable alkenyldiarylmethane non-nucleoside reverse transcriptase inhibitors incorporating N-methoxy imidoyl halide and 1,2,4-oxadiazole systems. *J Med Chem* 2007;50(14):3314–21.
- [139] Deng BL, Hartman TL, Buckheit Jr RW, Pannecouque C, De Clercq E, Cushman M. Replacement of the metabolically labile methyl esters in the alkenyldiarylmethane series of non-nucleoside reverse transcriptase inhibitors with isoxazolone, isoxazole, oxazolone, or cyano substituents. *J Med Chem* 2006;49(17):5316–23.
- [140] Watson KG, Brown RN, Cameron R, Chalmers DK, Hamilton S, Jin B, et al. An orally bioavailable oxime ether capsid binder with potent activity against human rhinovirus. *J Med Chem* 2003;46(15):3181–4.
- [141] Kozikowski AP, Roberti M, Xiang L, Bergmann JS, Callahan PM, Cunningham KA, et al. Structure-activity relationship studies of cocaine: replacement of the C-2 ester group by vinyl argues against H-bonding and provides an esterase-resistant, high-affinity cocaine analogue. *J Med Chem* 1992;35:4764–6.
- [142] Lipinski CA. Bioisosterism in drug design. In: Bailey DM, editor. Annual report in medicinal chemistry, vol. 21. San Diego: Academic Press; 1986. p. 283–91.
- [143] Wermuth CG. Aminopyridazines: an alternate route to potent muscarinic agonists with no cholinergic syndrome. *II Farmaco* 1993;48:253–74.
- [144] Huff JR, Anderson PS, Baldwin JJ, Clineschmidt BV, Guare JP, Lotti VJ, et al. N-(1,3,4,6,7,12b-hexahydro-2H-benzo[b]furo[2,3-a]quinolin-2-yl)-N-methyl-2-hydroxyethane-sulfonamide: a potent and selective α_2 -adrenoreceptor antagonist. *J Med Chem* 1985;28:1756–9.
- [145] Spatola AF. Peptide backbone modifications: structure-activity analysis of peptides containing amide bond surrogates. In: Weinstein B, editor. Chemistry and biochemistry of amino acids, peptides, and proteins, vol. 7. New York: Marcel Dekker; 1983. p. 267–357.
- [146] Fauchère J-L. Elements for the rational design of peptide drugs. In: Testa B, editor. Advances in drug research, vol. 15. London: Academic Press; 1986. p. 29–69.
- [147] Fournier J-P, Moreau RC, Narcisse G, Choay P. Synthèse et propriétés pharmacologiques de sulfonylurées isostères du glibenclamide [Synthesis and pharmacological properties of isosteric sulfonylureas glibenclamide]. *Eur J Med Chem* 1982;17:81–4 [in French].
- [148] Smith III AB, Holcomb RC, Guzman MC, Keenan TP, Sprengeler PA, Hirschmann R. An effective synthesis of scalemic 3,5,5-trisubstituted pyrrolin-4-ones. *Tetrahedron Lett* 1993;34:63–6.
- [149] Smith III AB, Keenan TP, Holcomb RC, Sprengeler PA, Guzman MC, Wood JL, et al. Design, synthesis, and crystal structure of a pyrrolinone-based peptidomimetic possessing the conformation of a β -strand: potential application to the design of novel inhibitors of proteolytic enzymes. *J Amer Chem Soc* 1992;114:10672–4.
- [150] Allmendinger T, Felder E, Hungerbuehler E. Fluoroolefin dipeptide isosteres. In: Weldi JT, editor. Selective fluorination in organic and bioorganic chemistry [Vol ACS Symp 456]. Washington: American Chemical Society; 1991. p. 186–95.
- [151] Chandrakumar NS, Yonan PK, Stapelfeld A, Svage M, Rorbacher E, Contreras PC, et al. Preparation and opioid activity of analogues of the analgesic dipeptide 2,6-dimethyl-1-tyrosyl-N-(3-phenylpropyl)-d-alanylamine. *J Med Chem* 1992;35:223–33.
- [152] Naylor EM, Colandrea VJ, Candelore MR, Cascieri MA, Colwell Jr LF, Deng L, et al. 3-Pyridylethanolamines: potent and selective human beta 3 adrenergic receptor agonists. *Bioorg Med Chem Lett* 1998;8(21):3087–92.
- [153] Naylor EM, Parmee ER, Colandrea VJ, Perkins L, Brockunier L, Candelore MR, et al. Human beta3 adrenergic receptor agonists containing imidazolidinone and imidazolone benzenesulfonamides. *Bioorg Med Chem Lett* 1999;9(5):755–8.
- [154] Feng DD, Biftu T, Candelore MR, Cascieri MA, Colwell Jr LF, Deng L, et al. Discovery of an orally bioavailable alkyl oxadiazole beta3 adrenergic receptor agonist. *Bioorg Med Chem Lett* 2000;10(13):1427–9.
- [155] Biftu T, Feng DD, Liang GB, Kuo H, Qian X, Naylor EM, et al. Synthesis and SAR of benzyl and phenoxymethylene oxadiazole benzenesulfonamides as selective beta3 adrenergic receptor agonist antiobesity agents. *Bioorg Med Chem Lett* 2000;10(13):1431–4.
- [156] Parmee ER, Naylor EM, Perkins L, Colandrea VJ, Ok HO, Candelore MR, et al. Human beta3 adrenergic receptor agonists containing cyclic ureidobenzenesulfonamides. *Bioorg Med Chem Lett* 1999;9(5):749–54.
- [157] Elzein E, Ibrahim P, Koltun DO, Rehder K, Shenk KD, Marquart TA, et al. CVT-4325: a potent fatty acid oxidation inhibitor with favorable oral bioavailability. *Bioorg Med Chem Lett* 2004;14(24):6017–21.
- [158] Koltun DO, Marquart TA, Shenk KD, Elzein E, Li Y, Nguyen M, et al. New fatty acid oxidation inhibitors with increased potency lacking adverse metabolic and electrophysiological properties. *Bioorg Med Chem Lett* 2004;14(2):549–52.
- [159] Borg S, Vollinga RC, Labarre M, Payza K, Terenius L, Luthman K. Design, synthesis, and evaluation of Phe-Gly mimetics: heterocyclic building blocks for pseudopeptides. *J Med Chem* 1999;42(21):4331–42.
- [160] Ladduwahetty T, Baker R, Cascieri MA, Chambers MS, Haworth K, Keown LE, et al. N-heteroaryl-2-phenyl-3-(benzyloxy)piperidines: a novel class of potent orally active human NK1 antagonists. *J Med Chem* 1996;39(15):2907–14.
- [161] Einsiedel J, Hubner H, Gmeiner P. Benzamide bioisosteres incorporating dihydroheteroazole substructures: EPC synthesis and SAR leading to a selective dopamine D4 receptor partial agonist (FAUC 179). *Bioorg Med Chem Lett* 2001;11(18):2533–6.
- [162] Einsiedel J, Hubner H, Gmeiner P. Cyclic amidines as benzamide bioisosteres: EPC synthesis and SAR studies leading to the selective dopamine D4 receptor agonist FAUC 312. *Bioorg Med Chem Lett* 2003;13(5):851–4.
- [163] Einsiedel J, Thomas C, Hubner H, Gmeiner P. Phenylloxazoles and phenylthiazoles as benzamide bioisosteres: synthesis and dopamine receptor binding profiles. *Bioorg Med Chem Lett* 2000;10(17):2041–4.
- [164] Thurkauf A, Yuan J, Chen X, He XS, Wasley JW, Hutchison A, et al. 2-Phenyl-4(5)-[[4-(pyrimidin-2-yl)piperazin-1-yl]methyl]imidazole. A highly selective antagonist at cloned human D4 receptors. *J Med Chem* 1997;40(1):1–3.
- [165] Vu CB, Corpuz EG, Merry TJ, Pradeepan SG, Bartlett C, Bohacek RS, et al. Discovery of potent and selective SH2 inhibitors of the tyrosine kinase ZAP-70. *J Med Chem* 1992;42(20):4088–98.
- [166] Clitherow JW, Beswick P, Irving WJ, Scopes DIC, Barnes JC, Clapham J, et al. Novel 1,2,4-oxadiazoles as potent and selective histamine H₃ receptor antagonists. *Bioorg Med Chem Lett* 1996;6:833–8.

II. LEAD COMPOUND DISCOVERY STRATEGIES

- [167] Swain CJ, Baker R, Kneen C, Moseley J, Saunders J, Seward EM, et al. Novel 5-HT₃ antagonists. Indole oxadiazoles. *J Med Chem* 1991;34(1):140–51.
- [168] Tully WR, Gardner CR, Gillespie RJ, Westwood R. 2-(Oxadiazolyl)- and 2-(thiazolyl)imidazo[1,2-a]pyrimidines as agonists and inverse agonists at benzodiazepine receptors. *J Med Chem* 1991;34(7):2060–7.
- [169] Orlek BS, Blaney FE, Brown F, Clark MS, Hadley MS, Hatcher J, et al. Comparison of azabicyclic esters and oxadiazoles as ligands for the muscarinic receptor. *J Med Chem* 1991;34(9):2726–35.
- [170] Fletcher SR, McIver E, Lewis S, Burkamp F, Leech C, Mason G, et al. The search for novel TRPV1-antagonists: from carboxamides to benzimidazoles and indazolones. *Bioorg Med Chem Lett* 2006;16(11):2872–6.
- [171] Wu WL, Burnett DA, Caplen MA, Domalski MS, Bennett C, Greenlee WJ, et al. Design and synthesis of orally efficacious benzimidazoles as melanin-concentrating hormone receptor 1 antagonists. *Bioorg Med Chem Lett* 2006;16(14):3674–8.
- [172] Wu WL, Burnett DA, Spring R, Qiang L, Sasikumar TK, Domalski MS, et al. Synthesis and structure-activity relationships of piperidine-based melanin-concentrating hormone receptor 1 antagonists. *Bioorg Med Chem Lett* 2006;16(14):3668–73.
- [173] Lanter JC, Fiordeliso JJ, Allan GF, Musto A, Hahn do W, Sui Z. A bioisosteric approach to the discovery of indole carbinol androgen receptor ligands. *Bioorg Med Chem Lett* 2006;16(21):5646–9.
- [174] Wouters J, Michaux C, Durant F, Dogne JM, Delarge J, Masereel B. Isosterism among analogues of torasemide: conformational, electronic and lipophilic properties. *Eur J Med Chem* 2000;35(10):923–9.
- [175] Luo G, Mattson GK, Bruce MA, Wong H, Murphy BJ, Longhi D, et al. Isosteric N-arylpiperazine replacements in a series of dihydropyridine NPY1 receptor antagonists. *Bioorg Med Chem Lett* 2004;14(24):5975–8.
- [176] Poindexter GS, Bruce MA, Breitenbucher JG, Higgins MA, Sit SY, Romine JL, et al. Dihydropyridine neuropeptide Y Y1 receptor antagonists 2. Bioisosteric urea replacements. *Bioorg Med Chem* 2004;12(2):507–21.
- [177] Lumma Jr WC, Anderson PS, Baldwin JJ, Bolhofer WA, Habecker CN, Hirshfield JM, et al. Inhibitors of gastric acid secretion: 3,4-diamino-1,2,5-thiadiazole 1-oxides and 1,1-dioxides as urea equivalents in a series of histamine H₂-receptor antagonists. *J Med Chem* 1982;25:207–10.
- [178] Young RC, Ganellin CR, Graham MJ, Grant EH. The dipole moments of 1,3-dimethylthiourea, 1,3-dimethyl-2-cyanoguanidine and 1,1-bis-methylamino-2-nitroethene in aqueous solution. *Tetrahedron* 1982;38:1493–7.
- [179] Young RC, Ganellin CR, Graham MJ, Roantree MJ, Grant EH. The dielectric properties of seven polar amidine-containing compounds of biological interest. *Tetrahedron Lett* 1985;26:1897–900.
- [180] Wright JL, Gregory TF, Kesten SR, Boxer PA, Serpa KA, Meltzer LT, et al. Subtype-selective N-methyl-D-aspartate receptor antagonists: synthesis and biological evaluation of 1-(heteroarylalkynyl)-4-benzylpiperidines. *J Med Chem* 2000;43:3408–19.
- [181] Wu WL, Burnett DA, Spring R, Greenlee WJ, Smith M, Favreau L, et al. Dopamine D1/D5 receptor antagonists with improved pharmacokinetics: design, synthesis, and biological evaluation of phenol bioisosteric analogues of benzazepine D1/D5 antagonists. *J Med Chem* 2005;48(3):680–93.
- [182] Burger A. Isosterism and bioisosterism in drug design. *Progr Drug Res* 1991;37:287–371.
- [183] Uloth RH, Kirk JR, Gould WA, Larsen AA. Sulfonanilides. I. Monoalkyl- and arylsulfonamidophenethanolamines. *J Med Chem* 1966;9(1):88–97.
- [184] Hacksell U, Arvidsson LE, Svensson U, Nilsson JL, Sanchez D, Wikstrom H, et al. 3-Phenylpiperidines. Central dopamine-autoreceptor stimulating activity. *J Med Chem* 1981;24(12):1475–82.
- [185] Bhaird NN, Fowler CJ, Thorberg O, Tipton KF. Involvement of catechol-O-methyl transferase in the metabolism of the putative dopamine autoreceptor agonist 3-PPP(3-(3-hydroxyphenyl)-N-n-propylpiperidine). *Biochem Pharmacol* 1985;34(19):3599–601.
- [186] Jaen JC, Wise LD, Caprathe BW, Teclé H, Bergmeier S, Humblet CC, et al. 4-(1,2,5,6-Tetrahydro-1-alkyl-3-pyridinyl)-2-thiazolamines: a novel class of compounds with central dopamine agonist properties. *J Med Chem* 1990;33(1):311–17.
- [187] Mierau J, Schneider FJ, Ensinger HA, Chio CL, Lajiness ME, Huff RM. Pramipexole binding and activation of cloned and expressed dopamine D₂, D₃ and D₄ receptors. *Eur J Pharmacol* 1995;290(1):29–36.
- [188] Schneider CS, Mierau J. Dopamine autoreceptor agonists: resolution and pharmacological activity of 2,6-diaminotetrahydrobenzothiazole and an aminothiazole analogue of apomorphine. *J Med Chem* 1987;30(3):494–8.
- [189] van Vliet LA, Rodenhuis N, Wikstrom H, Pugsley TA, Serpa KA, Meltzer LT, et al. Thiazolindans and thiazolobenzopyrans: a novel class of orally active central dopamine (partial) agonists. *J Med Chem* 2000;43(19):3549–57.
- [190] Blum G, Gazit A, Levitzki A. Development of new insulin-like growth factor-1 receptor kinase inhibitors using catechol mimics. *J Biol Chem* 2003;278(42):40442–54.
- [191] Hubner H, Haubmann C, Utz W, Gmeiner P. Conjugated enynes as nonaromatic catechol bioisosteres: synthesis binding experiments, and computational studies of novel dopamine receptor agonists recognizing preferentially the D subtype. *J Med Chem* 2000;43(4):756–62.
- [192] Lenz C, Boeckler F, Hubner H, Gmeiner P. Analogues of FAUC 73 revealing new insights into the structural requirements of nonaromatic dopamine D₃ receptor agonists. *Bioorg Med Chem* 2004;12(1):113–17.
- [193] Lenz C, Haubmann C, Hubner H, Boeckler F, Gmeiner P. Fancy bioisosteres: synthesis and dopaminergic properties of the endiynes FAUC 88 as a novel non-aromatic D₃ agonist. *Bioorg Med Chem* 2005;13(1):185–91.
- [194] Street LJ, Baker R, Davey WB, Guiblin AR, Jelley RA, Reeve AJ, et al. Synthesis and serotonergic activity of N,N-dimethyl-2-[5-(1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethylamine and analogues: potent agonists for 5-HT_{1D} receptors. *J Med Chem* 1995;38(10):1799–810.
- [195] Glen RC, Martin GR, Hill AP, Hyde RM, Woollard PM, Salmon JA, et al. Computer-aided design and synthesis of 5-substituted tryptamines and their pharmacology at the 5-HT_{1D} receptor: discovery of compounds with potential anti-migraine properties. *J Med Chem* 1995;38(18):3566–80.
- [196] Habeeb AG, Praveen Rao PN, Knaus EE. Design and synthesis of celecoxib and rofecoxib analogues as selective cyclooxygenase-2 (COX-2) inhibitors: replacement of sulfonamide and methylsulfonyl pharmacophores by an azido bioisostere. *J Med Chem* 2001;44(18):3039–42.
- [197] Larson AA, Lish PM. A new bio-isostere: alkylsulphonamidophenethanolamines. *Nature (London)* 1964;203:1283–5.

II. LEAD COMPOUND DISCOVERY STRATEGIES

- [198] Roderick SL, Fournié-Zaluski MC, Roques BP, Matthews BW. Thiorphan and retro-thiorphan display equivalent interactions when bound to crystalline thermolysin. *Biochemistry* 1989;28:1493–7.
- [199] Büchi J, Stünzi E, Flury M, Hirt R, Labhart P, Ragaz L. Über lokalanästhetisch wirksame basische ester und amide verschiedener alkoxy-amino-benzoesäuren [Concerning local anesthetic effective basic esters and amides of different alkoxy-amino-benzoic acids]. *Helv Chim Acta* 1951;34:1002–13 [in German].
- [200] Bös M, Jenck F, Martin JR, Moreau J-L, Sleight AJ, Wichmann J, et al. Novel agonists of 5HT_{2C} receptors. Synthesis and biological evaluation of substituted 2-(indol-1-yl)-1-methylethylamines and 2-(indeno[1,2-b]pyrrol-1-yl)-1-methylethylamines. Improved therapeutics for obsessive compulsive disorder. *J Med Chem* 1997;40:2762–9.
- [201] Wermuth CG. Selective optimization of side activities: another way for drug discovery. *J Med Chem* 2004;47(6):1303–14.
- [202] Böhm H-J, Flohr A, Stahl M. Scaffold hopping. *Drug Disc Today Technol* 2004;1(3):217–24.
- [203] Schneider G, Schneider P, Renner S. Scaffold hopping: how far can you jump? *QSAR Comb Sci* 2006;25(12):1162–71.
- [204] Low CM, Buck IM, Cooke T, Cushnir JR, Kalindjian SB, Kotecha A, et al. Scaffold hopping with molecular field points: identification of a cholecystokinin-2 (CCK₂) receptor pharmacophore and its use in the design of a prototypical series of pyrrole- and imidazole-based CCK₂ antagonists. *J Med Chem* 2005;48(22):6790–802.
- [205] Huang D, Luthi U, Kolb P, Edler K, Cecchini M, Audetat S, et al. Discovery of cell-permeable non-peptide inhibitors of beta-secretase by high-throughput docking and continuum electrostatics calculation. *J Med Chem* 2005;48(16):5108–11.
- [206] Steindl TM, Crump CE, Hayden FG, Langer T. Pharmacophore modeling, docking, and principal component analysis based clustering: combined computer-assisted approaches to identify new inhibitors of the human rhinovirus coat protein. *J Med Chem* 2005;48(20):6250–60.
- [207] Wilhelm M. The chemistry of polycyclic psycho-active drugs: serendipity or systematic investigation? *Pharm J* 1975;214:414–16.
- [208] Yoshimura H, Kikuchi K, Hibi S, Tagami K, Satoh T, Yamauchi T, et al. Discovery of novel and potent retinoic acid receptor alpha agonists: syntheses and evaluation of benzofuran-yl-pyrrole and benzothiophen-yl-pyrrole. *J Med Chem* 2000;43:2929–37.
- [209] Chenoweth MB, McCarthy LP. On the mechanism of the pharmacophoric effect of halogenation. *Pharmacol Rev* 1963;15:673–707.
- [210] Goldman P. The carbon–fluorine bond in compounds of biological interest. *Science* 1969;164:1123–30.
- [211] Peters RA. Biochemical lesions and lethal synthesis. Oxford: Pergamon Press; 1963.
- [212] Chapman NB, James JW, Graham JDP, Lewis GP. Chemical reactivity and pharmacological activity among 2-haloethylamine derivatives with a naphthylmethyl group. *Chem Ind (London)* 1952;805–7.
- [213] Vaughan JRJ, Anderson GW, Clapp RC, Clark JH, English JP, Howard KL, et al. Antihistamine agents. IV. Halogenated N,N-dimethyl-N⁹-benzyl-N-(2-pyridyl)-ethylenediamines. *J Org Chem* 1949;14:228–34.
- [214] Friedman HL. In: Influence of isosteric replacements upon biological activity, Symposium on chemical–biological correlation, Washington, D.C.: National Research Council; 1951. p. 295.
- [215] Schatz VB. Isosterism and bioisosterism. In: Burger A, editor. Medicinal chemistry. New York: Interscience Publishers, Inc.; 1963. p. 72–88.
- [216] Fessenden RJ, Fessenden JS. The biological properties of silicon compounds. In: Harper NJ, Simmonds AB, editors. Advances in drug research, vol. 4. London: Academic Press; 1967. p. 95–132.
- [217] Tacke R, Zilch H. Sila-substitution-a useful strategy for drug design? *Endeavour New Series* 1986;10:191–7.
- [218] Tacke R, Zilch H. Drug-design by sila-substitution and microbial transformations of organosilicon compounds: some recent results. *L'Actualité Chimique* 1986;3:75–82.
- [219] Ricci A, Seconi G, Taddei M. Biororganosilicon chemistry: trends and perspectives. *Chimica Oggi-Chemistry Today* 1989;7:15–21.
- [220] Showell GA, Mills JS. Chemistry challenges in lead optimization: silicon isosteres in drug discovery. *Drug Disc Today* 2003;8(12):551–6.
- [221] Anonymous. Zifrosilone. *Drugs Fut* 1994;19:854–5.
- [222] Hornsperger J-M, Collard J-N, Heydt JG, Giacobini E, Funes S, Dow J, et al. Trimethylsilylated trifluoromethyl ketones, a novel class of acetylcholinesterase inhibitors: biochemical and pharmacological profile of MDL 73,745. *Biochem Soc Trans* 1994;22:758–63.
- [223] Metcalf RL, Fukuto TR. Silicon-containing carbamate insecticides. *J Econ Entomol* 1965;58:1151.
- [224] Fessenden RJ, Coon MD. Silicon-substituted medicinal agents. Silacarbamates related to meprobamate. *J Med Chem* 1965;8:604–8.
- [225] Tacke R. Sila-Pharmaka, XIX. Sila-Pridinol und Pridinol: darstellung und Eigenschaften sowie Strukturen im kristallinen und gelösten Zustand [Sila-pharmaceutics, XIX. Sila pridinol and pridinol: preparation and properties and structures in the crystalline and dissolved state]. *Chem Ber* 1980;113:1962–80 [in German].
- [226] Moberg WK. Fungicidal 1,2,3-triazole derivatives. US Patent 4,510,136 (Apr 9, 1985 to EI Du Pont de Nemours & Co); 1985.
- [227] Damour D, Barreau M, Dutruc-Rosset G, Doble A, Piot O, Mignani S. 1,1-Diphenyl-3-dialkylamino-1-silacyclopentane derivatives: a new class of potent and selective 5-HT_{2A} antagonists. *Bioorg Med Chem Lett* 1994;4:415–20.
- [228] Gotteland J-P, Brunel I, Gendre F, Désiré J, Delhon A, Junquéro D, et al. (Aryloxy)methylsilane derivatives as new cholesterol biosynthesis inhibitors: synthesis and hypocholesterolemic activity of a new class of squalene epoxidase inhibitors. *J Med Chem* 1995;38:3207–16.
- [229] Gotteland J-P, Delhon A, Junquéro D, Oms P, Halazy S. Design and synthesis of new hypocholesteremic organosilanes with antioxidant properties. *Bioorg Med Chem Lett* 1996;6:533–8.
- [230] Heinonen P, Sipilä H, Neuvonen K, Lönnberg H, Cockroft VB, Wurster S, et al. Synthesis and pharmacological properties of 4(5)-(2-ethyl-2,3-dihydro-2-silainden-2-yl)imidazole, a silicon analogue of atipamezole. *Eur J Med Chem* 1996;31:725–9.
- [231] Bom D, Curran DP, Kruszewski S, Zimmer SG, Strode JT, Kohlhaagen G, et al. The novel silatecan 7-tert-butyl-dimethylsilyl-10-hydroxycamptothecin displays high lipophilicity, improved human blood stability, and potent anticancer activity. *J Med Chem* 2000;43:3970–80.
- [232] Mutahi M, Nittoli T, Guo L, Sieburth SM. Silicon-based metalloprotease inhibitors: synthesis and evaluation of silanol and silanediol peptide analogues as inhibitors of angiotensin-converting enzyme. *J Am Chem Soc* 2002;124(25):7363–75.
- [233] Chen CA, Sieburth SM, Glekas A, Hewitt GW, Trainor GL, Erickson-Viitanen S, et al. Drug design with a new transition state analog of the hydrated carbonyl: silicon-based inhibitors of the HIV protease. *Chem Biol* 2001;8(12):1161–6.
- [234] Showell GA, Barnes MJ, Daiss JO, Mills JS, Montana JG, Tacke R, et al. (R)-sila-venlafaxine: a selective noradrenaline reuptake inhibitor for the treatment of emesis. *Bioorg Med Chem Lett* 2006;16(9):2555–8.

II. LEAD COMPOUND DISCOVERY STRATEGIES

- [235] Barnes MJ, Conroy R, Miller DJ, Mills JS, Montana JG, Pooni PK, et al. Trimethylsilylpyrazoles as novel inhibitors of p38 MAP kinase: a new use of silicon bioisosteres in medicinal chemistry. *Bioorg Med Chem Lett* 2007;17(2):354–7.
- [236] Sommer LH, Bennet OF, Campbell PG, Weyenberg DR. Stereochemistry of hydride ion displacement from silicon. Enhanced rates at bridgehead and 4-ring silicon atoms. *J Amer Chem Soc* 1957;79:3295–6.
- [237] Alam F, Soloway AH, Bapat BV, Barth RF, Adams DM. Boron compounds for neutron capture therapy. *Basic Life Sci* 1989;50:107–11.
- [238] Gabel D. Tumor-seeking for boron neutron capture therapy: synthesis and biodistribution. *Basic Life Sci* 1989;50:233–41.
- [239] Kahl SB, Joel DD, Finkel GC, Micca PL, Nawrocky MM, Coderre JA. A carboranyl porphyrin for boron neutron capture therapy of brain tumors. *Basic Life Sci* 1989;50:193–203.
- [240] Caujolle F, Chan PH. Structure chimique et activité spasmolytique des organoboriques [Chemical structure and spasmolytic activity of organo-boric compounds]. *Arch Int Pharmacodyn Ther* 1968;172:467–74 [in French].
- [241] Mubarak SIM, Stanford JB, Sugden JK. Some aspects of the antimicrobial and chemical properties of phenyl boronate esters of chloramphenicol. *Drug Dev Ind Pharm* 1984;10:1131–60.
- [242] Hutter R, Keller-Schierlein W, Knusel F, Prelog V, Rodgers Jr GC, Suter P, et al. The metabolic products of microorganisms. Boromycin. *Helv Chim Acta* 1967;50(6):1533–9.
- [243] Düinitz JD, Hawley DM, Miklos D, White DNJ, Berlin Y, Marusik R, et al. Structure of boromycin. *Helv Chim Acta* 1971;54:1709–13.
- [244] Okami Y, Okazaki T, Kitahara T, Umezawa H. Studies on marine microorganisms. V. A new antibiotic, aplasmomycin, produced by a streptomycete isolated from shallow sea mud. *J Antibiot (Tokyo)* 1976;29(10):1019–25.
- [245] Kinder DH, Katzenellenbogen JA. Acylamino boronic acids and difluoroborane analogues of amino acids: potent inhibitors of chymotrypsin and elastase. *J Med Chem* 1985;28:1917–25.
- [246] Adams J, Behnke M, Chen S, Cruickshank AA, Dick LR, Grenier L, et al. Potent selective inhibitors of the proteasome: dipeptidyl boronic acids. *Bioorg Med Chem Lett* 1988;8(4):333–8.
- [247] Dembitsky VM, Srebnik M. Synthesis and biological activity of α -aminoboronic acids, amine-carboxyboranes, and their derivatives. *Tetrahedron* 2003;59(5):579–93.
- [248] Surolia N, RamachandraRao SP, Surolia A. Paradigm shifts in malaria parasite biochemistry and anti-malarial chemotherapy. *Bioessays* 2002;24(2):192–6.
- [249] Jabbour A, Steinberg D, Dembitsky VM, Moussaieff A, Zaks B, Srebnik M. Synthesis and evaluation of oxazaborolidines for antibacterial activity against streptococcus mutans. *J Med Chem* 2004;47(10):2409–10.
- [250] Kumar SK, Hager E, Pettit C, Gurulingappa H, Davidson NE, Khan SR. Design, synthesis, and evaluation of novel boronic-chalcone derivatives as antitumor agents. *J Med Chem* 2003;46(14):2813–15.
- [251] Landauer W. On the chemical production of developmental abnormalities and of phenocopies in chicken embryos. *J Cell Comp Physiol* 1954;43(1):261–305.
- [252] Landauer W, Clark EM. On the role of riboflavin in the teratogenic activity of boric acid. *J Expt Zool* 1964;156:307–12.
- [253] Browning E. Toxicity of industrial metals. 2nd ed. New York: Appleton-Century-Crofts; 1969. p. 90–97.
- [254] Klayman DL, Günther WHH. Organic selenium compounds: their chemistry and biology. New York: Wiley-Interscience; 1973.
- [255] Kang S-I, Spears CP. Linear free energy relationships and cytotoxicities of para-substituted 2-haloethyl aryl selenides and bis-(chloroethyl) selenides. *J Med Chem* 1987;30:597–602.
- [256] Fischer H, Terlinden R, Löhr JP, Römer A. A novel biologically active selenoorganic compound. VIII. Biotransformation of ebselen. *Xenobiotica* 1988;18:1347–59.
- [257] Parnham MJ, Graf E. Seleno-organic compounds and the therapy of hydroperoxide-linked pathological conditions. *Biochem Pharmacol* 1987;36:3095–102.

II. LEAD COMPOUND DISCOVERY STRATEGIES

Conformational Restriction and Steric Hindrance in Medicinal Chemistry

Peter Wipf¹, Erin M. Skoda¹ and André Mann²

¹Department of Chemistry, University of Pittsburgh, Pittsburgh PA, USA; ²LAAS-CNRS, Université Louis Pasteur, Strasbourg, France

OUTLINE

I. Introduction	279	B. Atropisomers	285
A. Theoretical Aspects of Ligand Binding	280	C. Peptidomimetics	288
B. Steric Constraints	283	D. Methyl Group Effects on Conformation	290
C. Conformational Analysis	283	E. Dihedral Angle Optimization	291
D. Steric Effects	284	F. Diversity-Oriented Synthesis	292
E. Rigid Compounds and Bioavailability	284	III. Summary and Outlook	297
II. Case Studies	285	References	297
A. The Study of Receptor Structure	285		

I. INTRODUCTION

For the practice of medicinal chemistry, an understanding of the thermodynamics of molecular recognition processes is essential. Noncovalent interactions between a ligand and a protein are mediated by several parameters. Ion–ion, dipole–dipole, and ion–dipole forces, hydrophobicity, hydrogen bonding, and shape complementarity play significant roles. The magnitude of the binding equilibrium is determined by the thermodynamic contributions of each of these interactions. When two molecules bind, their binding energy must compensate for their desolvation—in most cases the removal of all or part of their water shell. The probability that a ligand is trapped in a receptor is dependent on the promotion of binding by favorable interactions of polar functional groups and the formation of hydrophobic contacts. In addition to desolvation, adverse factors for binding are mainly related to the restriction of translational and vibrational energies upon complex formation [1–4]. While the ligand generally loses conformational freedom, the entropy of the solvent increases. In order to overcome entropy penalties and improve the binding affinity of a ligand, a suitable strategy is to restrict its conformational flexibility. Therefore, once a lead compound has been identified for a targeted biological receptor, the optimization toward potency and/or selectivity usually involves the modification of its conformation. Depending on the chemical structure of the identified lead, conformational restriction and/or steric hindrance can be introduced by adding or removing key bonds and substituents. An optimization of the free energy released during the association of a ligand with a receptor can be achieved by modifying the spatial disposition of functional groups through steric strain, hybridization, or cyclization. The expected benefits are receptor selectivity, potency increase, pharmacophore optimization, and metabolic stabilization. As a bonus, this strategy can be expected to produce original compounds and innovative chemistry. Finally, there are also some risks: any structural change

in a compound alters more than one property and can have unexpected consequences. Indeed, this approach is valuable in cases where the lead compounds are exhibiting flexibility and where the low-energy conformations are not representative for a good fit to the receptor. In this chapter, some theoretical points related to the thermodynamic aspects of lead optimization will be addressed, and then several examples will be presented where the modification of rigidity has had an impact in drug design.

A. Theoretical Aspects of Ligand Binding

Molecular recognition is a key factor in all biological processes. Accordingly, many of the important issues in medicinal chemistry hinge on an understanding of the noncovalent interactions between a biomacromolecule (receptor) and a small ligand (drug). Under equilibrium conditions, the binding affinity can be expressed in terms of the difference in free energy (ΔG°) of free and bound states or the equilibrium constant (K), which are correlated by the Gibbs-van't Hoff equation (Eq. 11.1):

$$\Delta G^\circ = -RT \ln K$$

{with $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$; $R = 8.13 \text{ J/mol/K}$ }

(11.1)

The more negative ΔG° , the stronger is the binding of the ligand to the bioreceptor (the binding constant is large). As a reminder, if a noncovalent association is expected, ΔG° must be negative and the Gibbs equation usually contains a negative value for ΔH° and a positive value for $T\Delta S^\circ$. If $\Delta G^\circ = -30 \text{ kJ/mol}$, the association constant is in the micromolar range. By using the Gibbs equation, we can distinguish between the effects of functional group interactions within the complex (ΔH°) and changes in the entropy upon binding ($T\Delta S^\circ$). The values of the standard free enthalpy ΔH° can serve as a quantitative indicator of the changes in intermolecular bond energies (mainly hydrogen bonding and van der Waals interactions) occurring during the binding, while the standard free entropy ΔS° is a good indicator of the reorganization in the solvation shell and the conformational changes during the same process. Only a negative ΔG° favors the equilibrium of the association. While in the formation of a covalent bond, ΔH° usually has the major contribution to ΔG° , in the case of a noncovalent interaction or equilibrium, the contributions by ΔH° and $T\Delta S^\circ$ to the free energy are often comparable. Given the large bond strengths of 300 to 500 kJ/mol for covalent bonds and the prevalence of the enthalpy term ($\Delta H^\circ \gg T\Delta S^\circ$), the much smaller energy of 1 to 60 kJ/mol released in a reversible interaction is composed of roughly equivalent enthalpy and entropy terms ($\Delta H^\circ \cong T\Delta S^\circ$) [5–8] (Figure 11.1).

The binding energy values for noncovalent associations are small and comparable to the solvation energies that oppose them in the binding event in aqueous media. Binding constants have been estimated by taking advantage of the Gibbs equation. Böhm used a set of interactions arising from experimentally determined binding constants resulting from the association of small organic molecules with proteins [10]. Five types of ΔG° contributions were identified (Table 11.1) and their values were extracted from a regression analysis. These values are generally used for a semi-quantitative evaluation of binding constants and can be applied in ligand optimization.

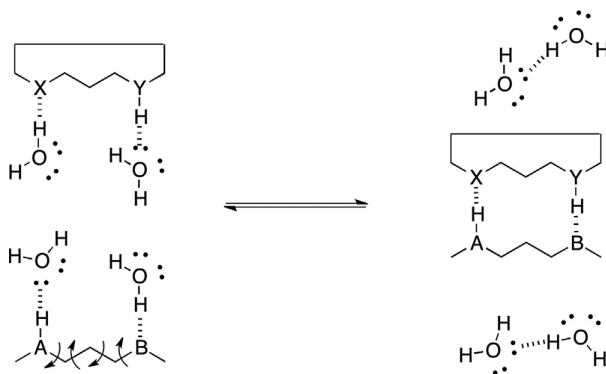


FIGURE 11.1 Noncovalent association between a ligand with two polar atoms (A and B) linked by a 3-carbon chain with a receptor containing complementary functions (X and Y), with the dashed line indicating hydrogen bonding [9].

TABLE 11.1 Average Values for ΔG° Contributions [9,10]

Physical process	ΔG° (kJ/mol)
Energy cost of bimolecular association	+5.4
Energy cost of restriction of an internal rotor	+2.0 (per rotor)
Hydrophobic effect (per Å of buried hydrocarbon)	−0.17/Å
Benefit of forming a neutral hydrogen bond of ideal geometry	−4.7
Benefit of forming an ionic hydrogen bond of ideal geometry	−8.3

1. Enthalpy–Entropy Compensation

A key factor that needs to be considered in the analysis of binding events is that a decrease in motion implies a decrease in entropy, since it results in fewer accessible arrangements. Consider the formation of a specific non-covalent bond (e.g., $L \cdots R$ for the transformation $L + R \rightarrow L \cdots R$). An increase in its strength (which corresponds to an additional negative contribution to ΔH° , favorable to the binding process) will be accompanied by an increasing restriction in the molecular motion of L and R in $L \cdots R$ (which corresponds to a decrease in ΔS° , unfavorable to the binding process). This opposing interplay between enthalpy and entropy is known as enthalpy–entropy compensation and represents a fundamental property of noncovalent interactions [11,12]. It arises because bonding opposes motion and, reciprocally, motion opposes bonding. The two effects can be compromised because the strength of noncovalent bonds at room temperature is comparable to kT , the product of the Boltzmann constant and the temperature. The thermodynamic parameters (ΔG° , ΔH° , ΔS°) for 136 ligands binding to ten biological receptors (mainly membrane receptors) have been analyzed [12]. It appeared that ΔH° and $T\Delta S^\circ$ values show a linear correlation (Eq. 11.2). This relationship seems remarkable in view of its high correlation and statistical relevance ($n = 186$). The observed enthalpy–entropy compensation seems to reflect general principles of the binding processes, irrespective of the nature of ligands and of their macromolecular targets:[12]

$$\Delta H^\circ = -40 \text{ kJ mol}^{-1} + 278 \Delta S^\circ (\text{in kJ K}^{-1} \text{mol}^{-1}) \quad (11.2)$$

$(n = 186, R^2 = 0.981, P \leq 0.001)$

This linear correlation reflects another concept: any tightening of the intermolecular bonds (the enthalpic contribution) is compensated by a loss of degrees of freedom (the entropic contribution). The binding association can be enthalpy- or entropy-driven. The origin of the $\Delta H/\Delta S$ compensation is probably related to an intrinsic property of the hydrogen bond, which determines the association of the participants (water, drug, and binding site) in the drug-receptor binding equilibrium [11,12]. Accordingly, the thermodynamic laws teach us that three factors are involved in improving binding affinity: (1) improving ligand-protein interactions over those with the solvent in order to obtain a favorable (negative) enthalpy change; (2) increasing the hydrophobicity of the ligand in order to displace solvent molecules in the binding site and increase entropy; and (3) pre-shaping the ligand to complement the geometry of the binding site in order to minimize the loss of conformational entropy upon binding [13].

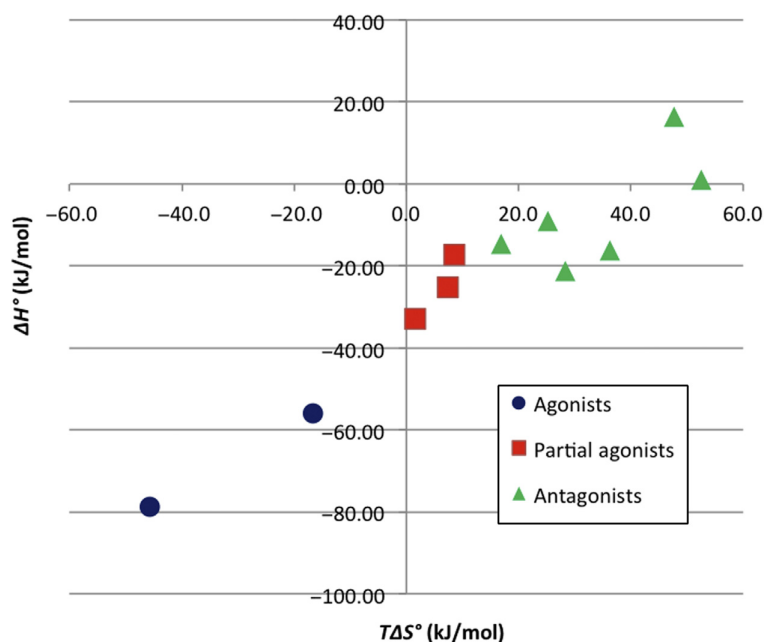
2. Enthalpy- Versus Entropy-Driven Binding

The following example is illustrative for the $\Delta H/\Delta S$ compensation. The binding of a series of agonists and antagonists of the β -adrenergic receptor displays thermodynamic differences between agonists and antagonists (Table 11.2) [14,15]. β -Adrenergic agonists and/or partial agonists are found to bind with large negative enthalpies (ΔH° from -79 to -17 kJ/mol), indicating strong electrostatic interactions between the bound conformation on the receptor, associated with a large loss of entropy (a tight complex is formed: $T\Delta S^\circ$ from -45.8 to $+8.6$ kJ/mol). Antagonists, on the other hand, do not fulfill the requirements of a good complementarity as evidenced by the criterion that their enthalpies of binding are small (ΔH° from -21.3 to $+16.4$ kJ/mol): The loss of entropy through conformational restriction is therefore less than that of the agonists, and—in fact—the entropy change is positive upon binding ($T\Delta S^\circ$ from $+16.9$ to $+52.5$ kJ/mol). Overall, agonists and antagonists have similar association constants, supporting the $\Delta H^\circ/\Delta S^\circ$ compensation paradigm (Table 11.2).

In order to improve our understanding of the $\Delta H^\circ/\Delta S^\circ$ compensation, the data from Table 11.2 are represented with a plot of ΔH° versus $T\Delta S^\circ$. Indeed, in Figure 11.2, clusters clearly differentiate agonists from antagonists or partial agonists. An interesting explanation based on the dimerization of the membrane-bound receptors (G-protein-coupled receptors) has been given by Williams:[8] the agonists (●; activating the receptor after

TABLE 11.2 Thermodynamic Parameters of Ligand Binding to the β -Adrenergic Receptor of Turkey Erythrocytes and Ratio of Equilibrium Constants K_D at 0°C and 37°C [14]

	ΔG° (kJ/mol)	ΔH° (kJ/mol)	ΔS° (J/mol deg)	$T\Delta S^\circ$ (kJ/mol)	K_D (37°C)/ K_D (0 °C)
AGONISTS					
(-)-isoproterenol	-39.3	-56.0	-54.0	-16.7	23
(-)-norephedrine	-33.1	-78.9	-147.7	-45.8	55
PARTIAL AGONISTS					
soteranol	-34.4	-32.8	+5.3	+1.6	5.3
fenoterol	-32.7	-25.4	+23.6	+7.3	3.6
terbutaline	-25.9	-17.3	+27.8	+8.6	2.4
ANTAGONISTS					
(-)-propranolol	-52.3	-16.1	+116.7	+36.2	2.6
IPS-339	-51.5	+1.1	+169.5	+52.5	0.95
pindolol	-49.6	-21.3	+91.2	+28.3	3.0
sotalol	-34.4	-9.0	+81.6	+25.3	1.6
atenolol	-31.3	-14.5	+54.4	+16.9	2.1
practolol	-31.2	+16.4	+153.6	+47.6	0.45

**FIGURE 11.2** Plot of ΔH° versus $T\Delta S^\circ$ for the binding of agonists (●), antagonists (▲), and partial agonists (■) to the β -adrenergic receptors. Data from Table 11.2 were used [14].

binding) induce receptor aggregation, which is beneficial in bonding (negative contribution to ΔH°) but adverse in entropy (negative contribution to $T\Delta S^\circ$). The antagonist (▲; without activation after binding) should therefore be (in comparison to agonist binding) relatively disfavoring for dimerization and entropy. The consequences are seen in Figure 11.2. The agonists (●) are binding to the β -receptors by an enthalpy-driven process, whereas the antagonists (▲) are binding with entropy and/or enthalpy–entropy-driven processes.

In contrast, if agonists induce dissociation of receptor oligomers (e.g., the adenosine A1 or A2 receptors) [8,16], antagonists may induce the formation of oligomers. Such antagonist binding should therefore be (in comparison to agonist binding) relatively favorable in overall enthalpy and unfavorable in entropy, in contrast to the agonists with adverse enthalpy and favorable entropy.

III. PRIMARY EXPLORATION OF STRUCTURE-ACTIVITY RELATIONSHIPS

Finally, an important point to stress is that hydrophobicity is a major source of binding in drug-receptor interactions. Based on a study of over 415 oral drugs, it appeared that on average drugs contain only one to two hydrogen donors and three to four acceptors, whereas the average number of hydrophobic atoms in a drug is 16 [17]. The contributions of polar and hydrophobic interactions in molecular recognition are related to the balance of enthalpy and entropy.

B. Steric Constraints

Before a small ligand interacts with a biological receptor, the two entities have their separate translational and rotational flexibility, which contribute to their respective entropy. Once the association occurs, some degrees of motion as well as internal rotations around single bonds are lost, and the consequence is an entropy cost of about -58 kJ/mol for a small ligand (MW < 1,000 Da) at room temperature [4,7,8]. As a general consequence of the binding of a ligand to a receptor, entropy is decreased due to the loss of degrees of motion when two molecules are rigidly constrained within a complex. The torsional contribution to the entropy (the free rotation of a bond) is related to the number of rotatable bonds in the ligand, and freezing one of them has a cost of 2–3 kJ/mol at room temperature [4,7,9]. Considering Equation 11.1, the entropy penalty will render ΔS negative, reducing the binding energy. In order for $\Delta G^\circ < 0$, the costs in reducing conformational flexibility must be offset by favorable intermolecular interactions such as hydrogen bonds, van der Waals packing, hydrophobic interactions, and Coulombic interactions. One consequence of this effect is that the binding optimization of a flexible ligand can be accomplished by making ΔH° more negative, by making ΔS° more positive, or by an appropriate combination of both. Theoretically, the highest value for a given binding constant is accessible to a flexible ligand if the receptor recognizes the ligand in its low-energy conformation that presents an optimal orientation of the functional groups. Therefore, all information gained about the active conformation of the ligand will serve the chemical design of a better lead structure. Some illustrative examples can be obtained from the incorporation of constrained amino acids into bioactive compounds [18]. However, since the determination of the thermodynamic parameters of ligand/receptor interactions is still in its infancy, optimizations in structure-based drug design rely on semi-quantitative and empirical strategies and structure–activity relationships (SAR). Conformational constraints, steric hindrance, and rigidification are frequently used principles in medicinal chemistry to explore binding parameters. Phenylalanine, a lipophilic amino acid, has been a popular substrate for evaluating this concept of rigidification. Nonnatural phenylalanine analogs have been synthesized by the introduction of bulky substituents, by the application of carbocyclic constraints, or by the incorporation of a functional group. Examples of conformationally constrained phenylalanine analogs are shown in Figure 11.3 [19–22].

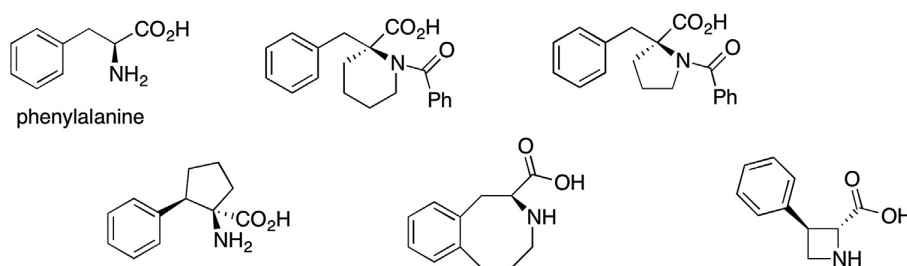


FIGURE 11.3 Conformational rigidification of phenylalanine analogs [19–22].

C. Conformational Analysis

Many computational methods are available to determine the low-energy conformations of a flexible ligand [23–25]. During the binding process, flexible ligands are refolded. This refolding is a general phenomenon and is presumably compensated by the search of a ligand for hydrogen bonds on the protein to replace the solute-solvent hydrogen bonds that are lost as the molecule enters the binding site (as shown in Figure 11.1) or in the unmasking of hydrophobic pockets on the receptor. A study has been performed on thirty-three ligands whose X-ray structures as well as conformations obtained by co-crystallization with their receptors have been recorded [26]. From this study, it appears that the degree of conformational change depends in part on the number of rotatable bonds in the ligand. For ligands with five or more rotatable bonds, the conformation in the crystal

structure does not represent the protein-bound conformation. Therefore, the solid-state structure of flexible ligands remains often of limited use for SAR analyses. Of course, in the more favorable cases, when a crystal structure of the complex–ligand–receptor can be obtained, the design of an improved ligand is facilitated because the main interactions can be recognized and then further optimized via synthesis.

D. Steric Effects

Steric effects may arise in a number of ways. Primary steric effects result from repulsions between valence electrons or nonbonded atoms. Such repulsions can only result in an increase in the energy of the system. In a chemical reaction, the overall steric effect may be either favorable or unfavorable. For example, if steric effects in the reactant are larger than in the product (or transition state), then the reaction is favored (steric augmentation); if the reverse case is true, the reaction is disfavored (steric diminution). The same arguments can be used in biological systems for the formation of a receptor–ligand complex. Comparing the binding of a ligand to a biological receptor with or without a subsequent chemical reaction—for example, an enzyme or a hormonal receptor—reveals some obvious differences. The enzymatic reactions involve only substructures in proximity to those atoms that are actually participating in bond making or breaking. Therefore, the enzyme tolerates structurally different ligands, provided the position on the substrate where the reaction should take place is accessible. Even if the direct affinity of a ligand for an enzyme is low, the subsequent chemical transformation can take place. In the formation of a ligand–receptor complex, any group of atoms that is in van der Waals contact with the receptor or the biomolecule can be or is involved in the binding event. If the receptor sits in a pocket that can adjust to any bioactive substance no matter its size or shape, then no steric effect will be observed. If, however, the parent biopolymer has limited conformational flexibility, and—as is likely—this flexibility is not equivalent in all directions, then a steric effect will be observed. Furthermore, the steric effect will be dependent on conformational states, and the minimal steric interaction principle will probably be observed. This principle states that a substituent whose steric effect is conformationally variable will prefer a conformation that minimizes steric repulsions and will give rise to the smallest steric strain. Finally, there are secondary steric effects on receptor binding that are produced by a substituent: (1) lowering the accessibility to an important group due to steric hindrance; (2) changing the population of a conformer due to steric effects; (3) shielding the active site from attack by a bulky group; and (4) variation in the electronic resonance of a π -bonded substituent by an out-of-plane repulsion.

E. Rigid Compounds and Bioavailability

Any effort toward improving bioavailability is of great importance in the drug discovery process. An intriguing correlation between the bioavailability of a compound and the number of its rotatable bounds was reported by Veber and coworkers using an empirical approach based on a set of 1,100 drug candidates and metabolism data collected from rats [27]. Remarkably, 10 or fewer rotatable bonds together with a polar surface area $<140 \text{ \AA}^2$ (or 12 or fewer H-bond donors and/or acceptors) irrespective of the molecular weight resulted in a high probability for good oral bioavailability in rats. This finding expanded the acceptable range of molecular weight values for a drug candidate beyond the threshold of 500 g/mol set by the Lipinski rules [28]. Veber suggested that by freezing some of the rotatable bounds, the molecular weight was no longer an essential parameter to be considered. The extent to which the number of rotatable bonds, as compared to other physicochemical parameters, affects oral bioavailability was recently also analyzed by Varma and coworkers [29]. Oral bioavailability (F) depends on the fraction of compound absorbed (F_a) as well as first-pass elimination that is determined by the fraction escaping gut-wall elimination (F_g) and the fraction escaping hepatic elimination (F_h), as shown in Equation 11.3. Each of the parameters evaluated (molecular weight, ionization state, lipophilicity, polar surface area, and rotatable bonds) affected at least one aspect of oral bioavailability. For example, while increasing molecular weight caused a decrease in F_a , increasing lipophilicity caused a decrease in F_g and F_h . Most notably, increasing the number of rotatable bonds had a negative effect on all three parameters and profoundly affected oral bioavailability [29]. Therefore, the introduction of conformational constraints in a drug candidate also has to be considered for resolving pharmacokinetic issues.

$$F = F_a \times F_g \times F_h \quad (11.3)$$

II. CASE STUDIES

A. The Study of Receptor Structure

A classic example of exploring the structure and binding requirements of a target enzyme by conformational restriction without the benefit of a co-crystal structure is presented by the μ -opioid receptor antagonists. Drugs that have been approved to target this receptor are used for pain management, and the name of the receptor family is derived from the prototypical antagonist of this family, morphine. Activation of the μ -opioid receptor family by endogenous opioid peptides in the body also affects behavioral and homeostatic functions and could therefore be used to treat indications in addition to pain. Zimmerman and coworkers first described the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine μ -opioid receptor antagonists in 1978 [30] and later clarified the structural requirements that lead to higher affinity binding (Figure 11.4, A) [31]. Subsequent to the discovery of these analogs, Le Bourdonnec and coworkers designed analogs of Zimmerman's antagonists in which the rotation about the N-substituent was constrained by the formation of a fused ring [32]. Because the stereochemical requirements at C3 and C4 had been established, the 4-stereoisomers of each of the regioisomers of the constrained analogs were synthesized. Interestingly both a potent antagonist (Figure 11.4, B) and agonist (Figure 11.4, C) were found among these analogs. An analysis of the lowest energy conformers of these three molecules showed good overlap of A and B, with the hydroxyphenyl substituent in the equatorial position. The lowest energy conformer of the agonist places the hydroxyphenyl substituent in the axial position. The crystal structure of the μ -receptor was not solved until 2012 [33], and the conformationally restricted analogs were useful in gaining information about the binding pocket of the receptor prior this discovery, providing a successful complement to the traditional bioactive structures based on morphine.

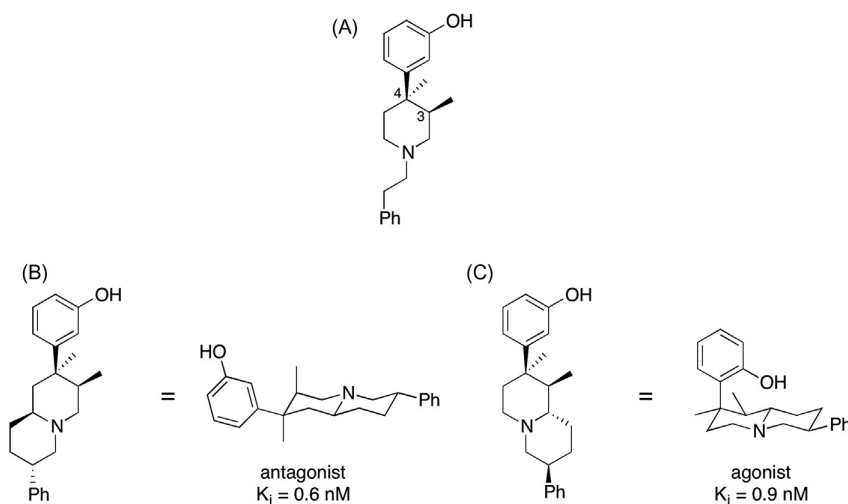


FIGURE 11.4 Structures of small molecules that interact with the μ -opioid receptor: (A) initial scaffold discovered [30,31] (B) constrained, potent antagonist; (C) constrained, potent agonist [32].

B. Atropisomers

Atropisomers are chiral due to restricted rotations of σ -bonds with barriers exceeding 20 kcal/mol, which results in a half-life of >1000 s at room temperature [34]. Atropisomers often result from a hindered rotation of bonds connecting two aromatic rings (as in Figure 11.5, A) or from a barrier in the ring flip of a medium-sized ring (as in Figure 11.5, B). Depending on the overall structure of the molecule, atropisomers can form either enantiomers or diastereomers. The first enantiomerically enriched atropisomers were isolated in 1922 [35], and since then differences in their physical properties and bioactivities have been noted [36–38].

Atropisomers racemize by a bond rotation, and the activation energy for this process depends on the electron distribution, solvent, temperature, and steric hindrance. Atropisomers that have a high barrier to racemization are easily separable and should be stable under physiological conditions. Conversely, atropisomers that have a low barrier to rotation will undergo interconversion under physiological conditions, and therefore it is unnecessary to separate them for biological activity differentiation and chemical characterization. Over the last two decades, the pharmaceutical industry has moved away from developing racemic mixtures as drug candidates; as a

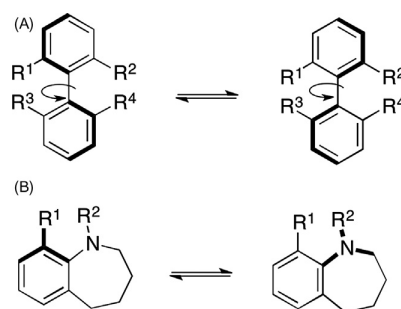


FIGURE 11.5 Generic atropisomers displaying (A) axial chirality due to hindered rotation of an aryl-aryl σ -bond and (B) planar chirality due to steric hindrance of the R groups and the dissymmetry of the bridging methylene and amine groups.

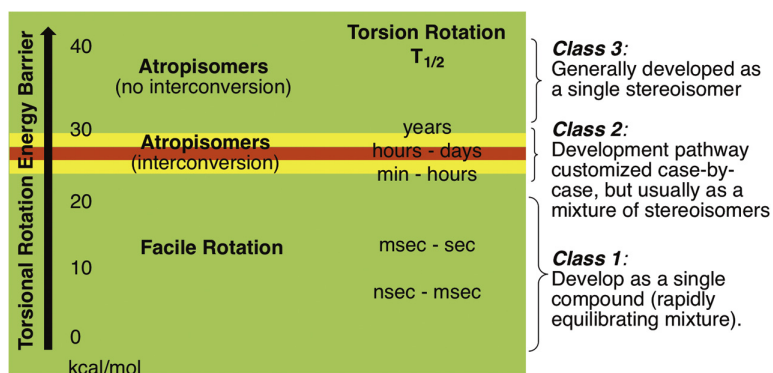


FIGURE 11.6 Table describing classes of atropisomers based on the barrier to rotation developed by LaPlante and coworkers for applications in drug development [36].

result, several reviews focus on the merits of developing a preclinical candidate as a single atropisomer rather than a mixture [36,37]. LaPlante and coworkers have established recommendations for the drug development of atropisomers based on US FDA guidelines summarized in Figure 11.6. [36,39]. In short, if the barrier to rotation of an atropisomer is high ($\Delta E_{\text{rot}} \geq 30$ kcal/mol, $t_{1/2}$ in years), then the atropisomers should be resolved and the less active isomer should be treated as an impurity (Figure 11.6, Class 3). If the atropisomers rapidly equilibrate ($\Delta E_{\text{rot}} < 20$ kcal/mol, $t_{1/2}$ of minutes, Class 1), they should be treated as a single compound, since separation and individual bioactivity assessment would be challenging. The gray area of atropisomer development lies within the ΔE_{rot} 20–30 kcal/mol range ($t_{1/2}$ of hours to days, Class 2). In this area, development is challenging due to difficulties in isolation and stability, and the authors recommend increasing the ΔE_{rot} by adjusting steric hindrance, if possible, to move these compounds into Class 3.

1. Bombesin Receptor Subtype-3 (BRS-3) agonists

Obesity is a growing problem in our society and has been shown to cause a number of other diseases, including type-2 diabetes, cardiovascular disease, cancer, and hypertension [40]. As the rate of obesity is increasing, so too is the need for an effective anti-obesity drug. It was found that metabolic defects and obesity are present in mice that lack functional bombesin receptor subtype-3 (BRS-3) [41,42], and a BRS-3 agonist caused a decrease in food intake accompanied by an increase in metabolic rate in diet-induced obese mice [43]. In a BRS-3 HTS screen, Merck chemists discovered the compound shown in Figure 11.7 (A) [44,45]. Follow-up chemistry led to the unexpected discovery that many members of this compound class existed as a mixture of two separable enantiomers (Figure 11.7, B and C). Upon biological testing, it was determined that the *R*-enantiomer was much less potent than the *S*-enantiomer, with IC_{50} values of 169 nM versus 1.4 nM, respectively.

To determine the extent of steric bulk necessary to allow a room temperature resolution of the atropisomers, several analogs were analyzed [45]. It was found that when the C-7 substituent was either an H or F atom, no atropisomerism was observed (Figure 11.8, A). Bulkier halogens, such as Cl, a hydroxyl group (Figure 11.8, A), or a fused ring (Figure 11.8, B), led to atropisomerism. As expected, when a pyridine nitrogen atom lone pair occupied the space at the 7-position, no atropisomerism was observed (Figure 11.8, C). The addition of a large

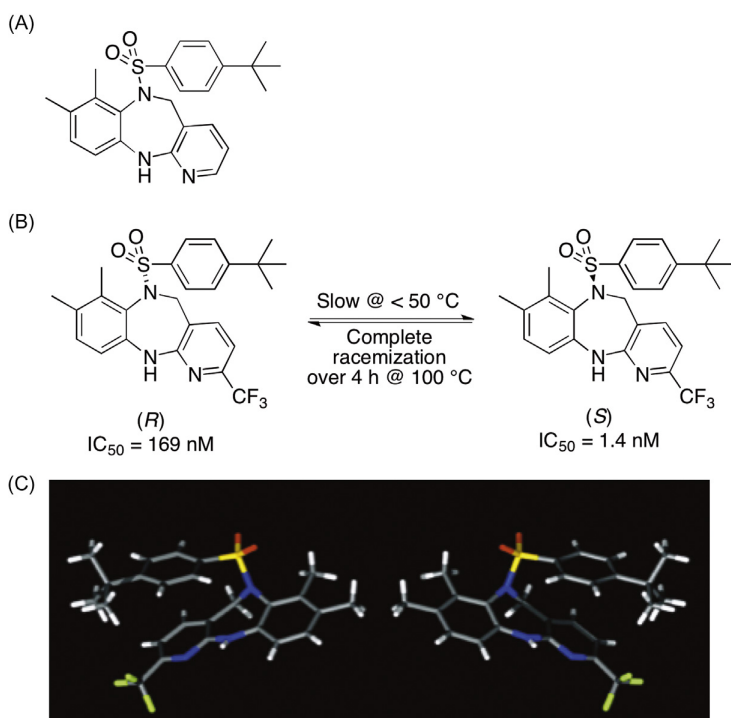


FIGURE 11.7 Structure of (A) an HTS hit from a BRS-3 screen, (B) more potent compounds resulting from follow-up chemistry that were separable atropisomers whose planar chirality was (C) confirmed by X-ray crystallography. Adapted with permission from Liu P, Lanza TJ, Chioda M, Jones C, Chobanian HR, Guo Y, et al. Discovery of benzodiazepine sulfonamide-based bombesin receptor subtype 3 agonists and their unusual chirality. *ACS Med Chem Lett* 2011;2:933–7. Copyright 2011 American Chemical Society.

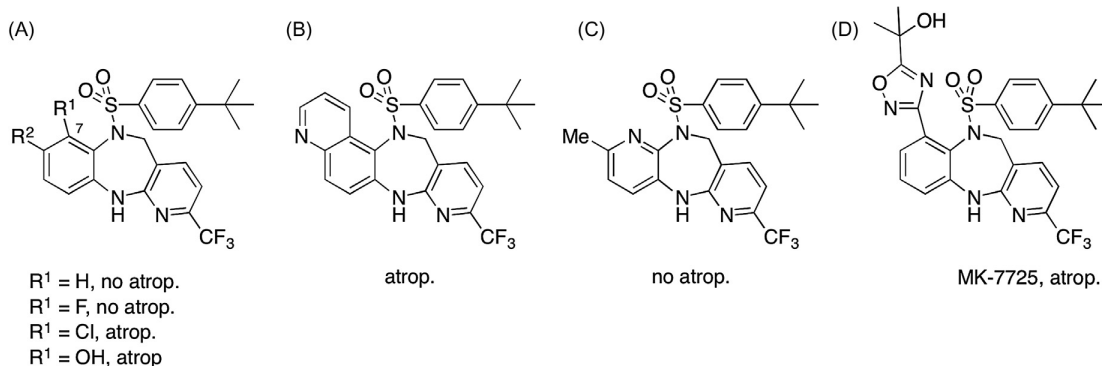


FIGURE 11.8 SAR study of atropisomers in which various substituents at C-7 were tested (no atrop. = no atropisomerism was observed; atrop. = atropisomerism was observed) [45].

heterocyclic substituent at C-7 led to the discovery of an inhibitor with comparable activity and greatly improved pharmacokinetics, MK-7725 (Figure 11.8, D). [46]

2. Glycine transporter 1 (GlyT1) inhibitors

Atropisomerism in the form of axial chirality is a more commonly observed occurrence, and enantiomers of this type were observed by Sugane and coworkers during their search for selective glycine transporter 1 (GlyT1) inhibitors [47]. GlyT1 is a glycine transporter that is present in forebrain areas where it terminates glycine activity by mediating its uptake [48,49]. It is believed that GlyT1 modulates NMDA receptor activity by regulating glycine levels, and a decrease in NMDA is a contributing factor to schizophrenia [50].

An HTS hit (Figure 11.9, A) from a GlyT1 screen was found to be a low micromolar inhibitor of GlyT1 but displayed no selectivity over the closely related GlyT2 [51]. It was noted that increasing the steric bulk at either the 3- or 4-position on the triazole ring increased the selectivity for GlyT1. Indeed, the *R*-enantiomer of the optimized

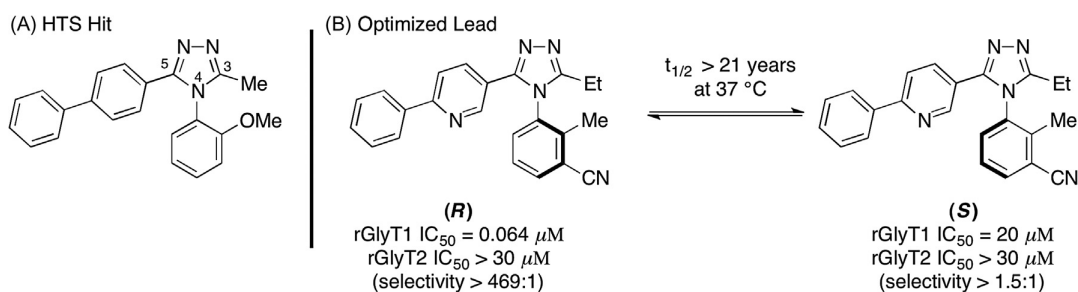


FIGURE 11.9 (A) Structure of an HTS hit from a GlyT1 screen; (B) optimized lead that resulted from increasing the steric bulk to impede axial rotation. [47].

lead (Figure 11.9, B) had an IC_{50} of 64 nM against GlyT1, while the *S*-enantiomer had an IC_{50} of 20 μM . These enantiomers were separable by chiral HPLC, and it was determined that the ΔE_{rot} was 31.4 kcal/mol (resulting in a $t_{1/2}$ of >21 years at 37°C), indicating that these compounds are Class 3 atropisomers (as described in Figure 11.6).

C. Peptidomimetics

1. Gramicidin S Derivatives

Gramicidin S (Figure 11.10) is a cyclic decapeptide natural product whose history spans more than seventy years. It displays antibiotic activity against both Gram-negative and Gram-positive bacteria by interacting with the bacterial lipid bilayer [52]. This interaction is closely linked to the structure of gramicidin S—its amphipathic properties are enhanced by its 3-dimensional scaffold. This enhancement is due to an antiparallel β -sheet that aligns hydrophilic and hydrophobic portions on opposite faces of the molecule. Two type II' β -turns and four intramolecular hydrogen bonds stabilize the extended secondary structure, which has been extensively studied

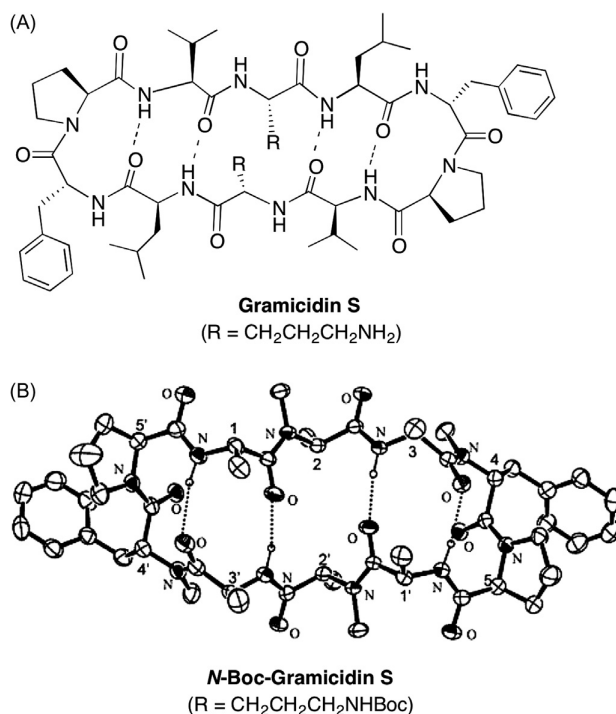


FIGURE 11.10 (A) Structure of gramicidin S showing the intramolecular hydrogen bonding that stabilizes the β -hairpin secondary structure; (B) X-ray structure of the Boc-derivative of gramicidin S [54].

by NMR [53], X-ray crystallography [54], and circular dichroism (CD) [55]. Perturbations to this 3-dimensional structure due to steric bulk or electronic variations that twist the molecule from its natural conformation cause a decrease or complete loss in function [56].

Because peptides are often plagued by poor bioavailability and rapid hydrolysis in vivo, bioisosteric replacements of the amide bond have been of great interest [57,58]. In order for such a rational design to be successful, the bioisostere must allow for a close match of the geometry of the peptide bond while remaining stable to peptidase cleavage. Among many isosteric substituents, the (*E*)-alkene peptide isosteres [58] have been quite successful, since they have a rigid geometry that is locked in place by a nonrotatable double bond and therefore mimic the sp^2 nature of the amide nitrogen particularly well. (*E*)-Alkene peptide isosteres are impervious to peptidase cleavage, but depending on the substituents attached to the double bond, any H-bonding in which an amide would participate can be lost. Furthermore, disubstituted alkenes are conformationally more flexible than the parent amide and display smaller dipole moments and greater lipophilicity. In the case of gramicidin S, loss of hydrogen bonds could be detrimental to the bioactivity, because they stabilize the molecule in its bioactive conformation. As such, this cyclopeptide antibiotic represents a useful probe substrate for the tolerance for bioisosteric group replacement and its effect on conformation and activity.

To explore the consequences of amide bond replacement on the properties of gramicidin S, more stable functional groups that should allow for the bioactive geometry of gramicidin S to remain intact were introduced into the backbone sequence. Specifically, (*E*)-alkene peptide isosteres with different substituents attached to the alkene were synthesized via a hydrozirconation/transmetalation/imine addition sequence. This synthetic sequence allowed for a stereoselective formation of the allylic amine portion of the isosteres from an alkyne and enantiomerically pure sulfinamide (Figure 11.11).

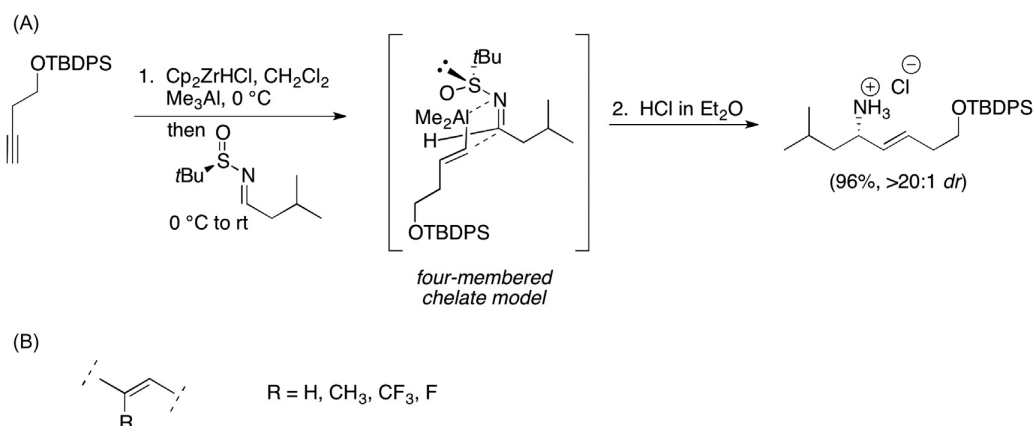


FIGURE 11.11 (A) Large-scale stereoselective synthesis of an allylic amine intermediate used in the synthesis of the (*E*)-alkene peptide isostere portion of the bioactive peptide mimic JP4-039 [59] and (B) examples of differentially substituted (*E*)-alkene peptide isosteres used in the gramicidin S analogs [60].

Trisubstituted alkenes with methyl, trifluoromethyl, and fluoro substituents as well as a disubstituted alkene were used as replacements for the Leu-Phe peptide bond. While the tertiary (*E*)-alkene peptide isostere containing the methyl substituent was of similar size as the corresponding trifluoromethyl analog, the methyl analog caused a greater perturbation of the secondary structure of the molecule, resulting in a CD spectrum that was indicative of a random coil secondary structure. The trifluoromethyl analog was a superior match of the parent compound in both solution and solid-state structures [55]. Even though the plane of the alkene was twisted 70° away from the interior of the β -turn due to the bulkiness and hydrophobicity of the CF_3 substituent, the overall geometry of the type II' β -turn was highly preserved, and the β -hairpin structure of the cyclopeptide was unchanged.

A deprotected derivative of a trisubstituted (*E*)-alkene peptide isostere was also a successful structural mimic of the D-Phe-Pro moiety. Not only did this molecule have the desired 3-dimensional structure in solution (as indicated by the CD spectrum), but it also demonstrated equipotent biological activity to GS against *Bacillus subtilis* (20 and $15\ \mu g/mL$, respectively) [60]. The fluoro- and disubstituted (*E*)-alkene peptide isosteres also formed β -sheets, successfully mimicking the natural peptide. Therefore, these molecules demonstrate that peptide bond isostere replacements can preserve both the 3-dimensional structure as well as the biological profile of the parent structure.

III. PRIMARY EXPLORATION OF STRUCTURE-ACTIVITY RELATIONSHIPS

2. XJB-5-131 and JP4-039 as GS-Mimics Targeting Mitochondria

Based on the properties of GS to interact with bacterial membranes and the knowledge that mitochondria evolved from bacteria, smaller and more drug-like GS-mimics were designed with the intent of using them to target the mitochondrial membrane. For the design of such a mimic, the ornithine side chains remained protected as the carbamate derivatives to decrease a potential hemolytic activity upon enrichment in erythrocytes. Furthermore, a segment of GS was used to decrease molecular weight, but the alkene peptide isostere and the D-Phe-Pro sequence were conserved to stabilize the type II' β -turn structure, decrease the potential for proteolytic degradation, and diminish the number of hydrogen bond donors and acceptors. The (*E*)-alkene isostere XJB-5-131 was found to match a type II' β -turn by CD analysis, and XJB-5-131 was enriched ca. 600-fold in mitochondria over the cytosol (Figure 11.12, A). The nitroxide “warhead” was introduced to react with reactive oxygen species (ROS) that are formed in the organelle, and the ROS scavenger XJB-5-131 proved to be effective in a number of disease and injury models involving cellular damage by reactive oxygen species. Such diseases include many acute and chronic degenerative disorders (e.g., hemorrhagic shock [61], hyperoxic acute lung injury [62], traumatic brain injury [63], microdialysis injury [64], and Huntington’s disease [65]).

Following the success of the GS-nitroxide XJB-5-131 as a mitochondrial-targeted ROS scavenging agent, attempts to generate a lower molecular weight analog led to the discovery of JP4-039 (Figure 11.12, B). The structure of JP4-039 contains a nitroxide directly attached to the dipeptide isostere, and it also adopts a type II' β -turn, as evidenced by its crystal structure [59]. A fluorescent derivative of JP4-039 was used to visualize the enrichment of this compound in mitochondria [66].

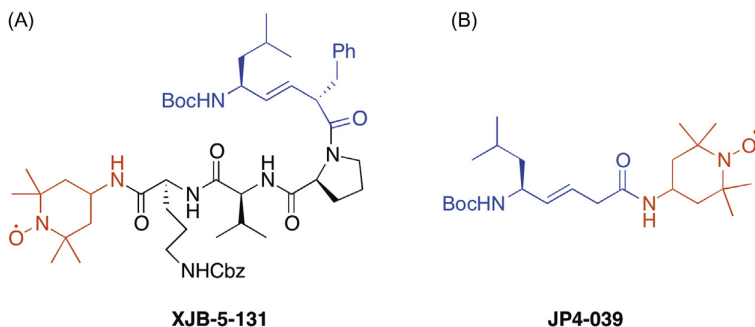


FIGURE 11.12 Structures of mitochondrial-targeted (A) XJB-5-131 and (B) JP4-039 (with the dipeptide mimetic in blue and the nitroxide in red).

D. Methyl Group Effects on Conformation

The seemingly innocuous methyl group is often added to small molecules to sterically block sites of metabolic activity and increase $t_{1/2}$. In some cases, its addition to a molecule also has a profound effect on biological potency [67,68]. Because the methyl group is a hydrophobic substituent, it can cause a slight increase in potency by reducing the desolvation energy that is required to remove the solvation by water molecules when the molecule enters a hydrophobic protein cavity from an aqueous environment [69,70]. This decrease in desolvation energy can increase the potency of a molecule by approximately 3.5-fold; any dramatic increase in potency beyond what is attributed to the increase in hydrophobicity is colloquially called the “magic methyl effect” [71] by medicinal chemists. Such “magic” effects have increased potency by over 1,000-fold [72] and even converted an agonist to an antagonist [73], but the addition of a methyl group is just as likely to decrease potency as it is to increase it [68].

1. Phospholipase D (PLD1/2) Inhibitors

Phospholipase D (PLD) catalyzes the hydrolysis of phosphatidylcholine into choline and phosphatidic acid, and suboptimal PLD function has been implicated in cancer [74] and CNS disorders [75,76]. By simply adding a methyl group to the ethylene diamine linker, Lindsley and coworkers were able to increase the activity of their PLD1 inhibitors from an IC_{50} of 11,800 nM to 20 nM (Figure 11.13) [77]. This dramatic increase in activity is likely due to a similar phenomenon observed in the factor Xa inhibitors [78]. The rotamer distribution of the dihedral angle around the amide backbone was analyzed, and it was found that when the methyl group was incorporated, fewer rotamers were formed and the rotational barriers increased. The more distinct conformational preferences favored the bioactive conformation [79].

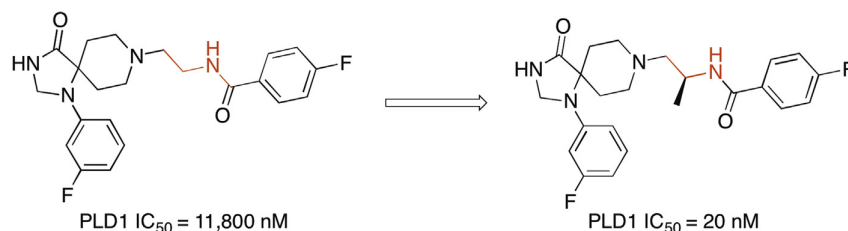


FIGURE 11.13 Dramatic effect on potency of PLD1 inhibitors by the addition of a methyl substituent, with the pertinent bonds shown in red [77].

2. Phosphatidylinositol-3-Kinase (PI3K) Inhibitors

While the PLD1 inhibitors experienced a dramatic increase in potency with the addition of a single methyl group, an analogous structural modification in Pfizer's phosphatidylinositol-3-kinase (PI3K) inhibitors had a much more variable effect on activity. The PI3K/mTOR (mammalian target of rapamycin) signaling pathway has been implicated in a number of disease pathologies including cancer [80] and aging [81]. Quinazolines, such as those shown in Figure 11.14 (A), were synthesized based on previously determined SAR [82]. It had been found that the C-2 methyl group was necessary for selectivity and was therefore incorporated into all new analogs. However, when a methyl group was added to the C-7 position (Figure 11.14, B), activity decreased substantially. Conversely, the addition of a methyl group at C-6 caused an almost 5-fold increase in potency over the 6-H derivative. These significant differences in potency with such seemingly small structural changes demonstrate the subtleties of relatively minor structural perturbations in medicinal chemistry. Computational studies of the 7-methyl and the 6-methyl derivatives showed that a planar orientation of the amide side-chain that was locked in place by the hydrogen bonding of the N-H to the quinazoline N-1 was optimal for the molecule to fit into the binding pocket [82]. The presence of the 7-methyl disrupts the planarity and introduces a twist to the N-C-C8-C9 dihedral angle calculated as $> 20^\circ$.

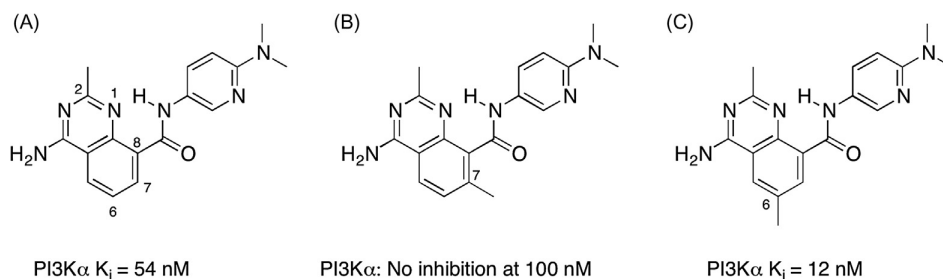
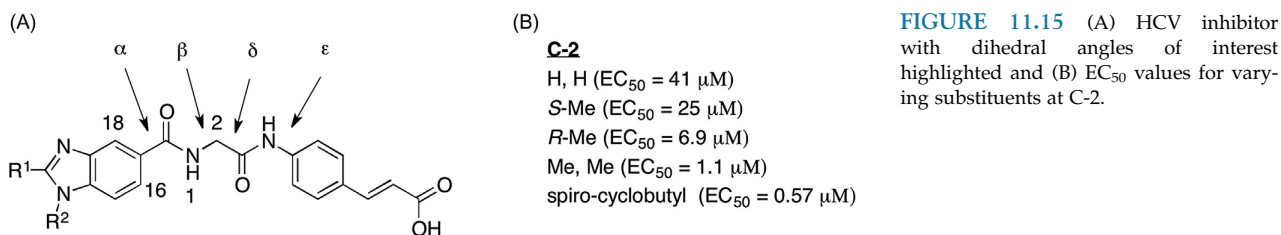


FIGURE 11.14 Structures of putative PI3K/mTOR inhibitors. The methyl group placement proved to be important for activity. (A) Absence of a methyl group at C-7 led to a fairly potent inhibitor with an IC_{50} of 54 nM, while (B) with a 7-methyl was inactive and (C) with a 6-methyl was the most potent inhibitor with an IC_{50} value of 12 nM [82].

E. Dihedral Angle Optimization

A thorough understanding of the receptor structure was used in the case of the detailed design of inhibitors of the hepatitis C virus (HCV). HCV is an infectious disease that primarily affects the liver with a chronic infection that eventually manifests itself by cirrhosis and possibly liver cancer or failure. The receptor structure of the virus was determined by a combination of X-ray crystallography of inhibitors bound to HCV NS5B, NMR spectroscopy, and docking [83]. This allowed for the detailed study of the bioactive conformation and specifically the requisite bond angles as shown in Figure 11.15 (A). Early in their studies, LaPlante and coworkers found that the bioactive conformation around dihedral angle α placed C-18 into the vicinity of NH-1. Several attempts to modulate this angle by adding a methyl group to C-16 or introducing a hydrogen-bonding pyridine N atom into the aromatic ring failed to improve potency, despite the bioactive conformation being supported by ROESY NMR correlations. Competing unfavorable forces such as electrostatic repulsion likely led to the undesirable potency. A more favorable outcome was obtained after modulating the rotation about the dihedral angles β and δ . Correlation of the cellular EC_{50} values with the substituents at C-2 showed that transitioning from the unsubstituted to a single methyl, a gem-dimethyl,



and finally a spiro-cyclobutyl group improved the cellular potency from $41 \mu M$ to $0.57 \mu M$ (Figure 11.15, B). Molecular mechanics was used to analyze the angle distributions of the unsubstituted C-2 compared to the spiro-cyclobutyl derivative, and the bioactive dihedral angle was more prevalent in the substituted analog.

The progression of molecular structure can be seen in Figure 11.16. The HTS hit (A) was active *in vitro* but had no cellular activity. Initial modification of the pertinent dihedral angle α led to the tryptophan series of compounds B. Unfortunately, this series had high micromolar cellular efficacy, which led to a transition to the diamide series (C). This series had the optimal dihedral angles for α , β , and δ , but still showed micromolar potency. Finally, dihedral angle ϵ was further restricted by the incorporation of the benzimidazole amide bond isostere as shown in D. This compound, with its fully optimized 3-dimensional structure, led to a dramatic improvement in cellular potency and served as the clinical candidate for the treatment of HCV infection.

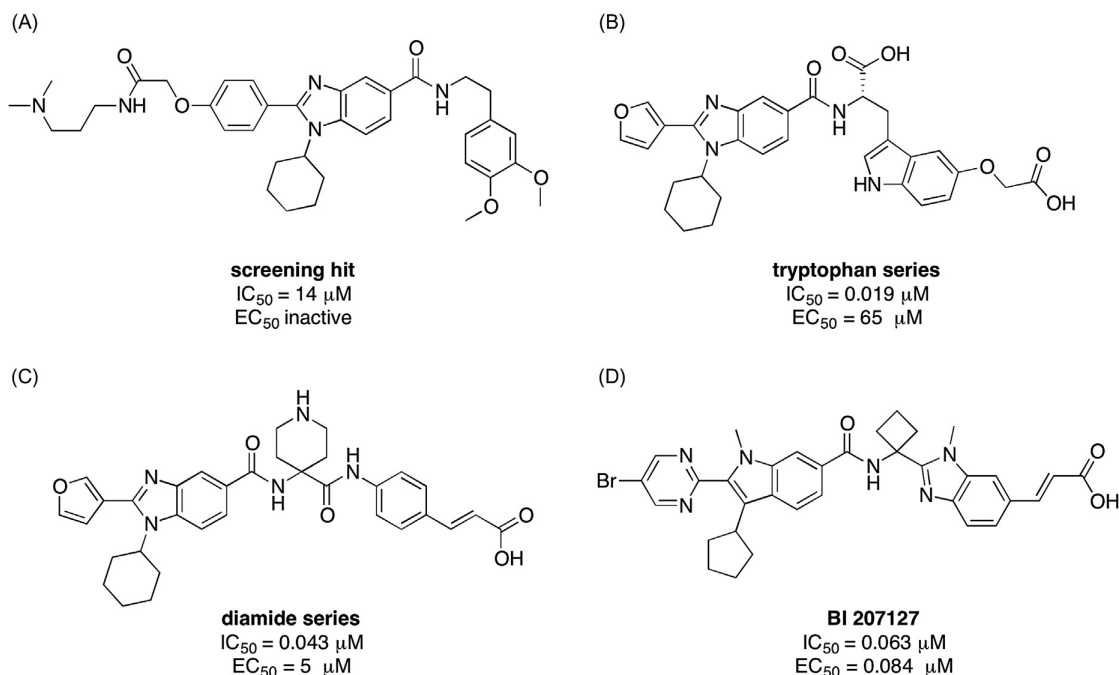


FIGURE 11.16 Structures of the (A) HTS hit, and (B) the lead structure initially used to determine bond angles, (C) the diamide series, and finally (D) the clinical candidate for HCV inhibition.

F. Diversity-Oriented Synthesis

Diversity-oriented synthesis (DOS) “involves the deliberate, simultaneous and efficient synthesis of more than one target compound in a diversity-driven approach to answer a complex problem.” [84] It is a strategy that allows for the use of an efficient synthetic design to explore chemical space. This is an important as well as challenging undertaking, since chemical space is vast and significantly underexplored [85]. Estimates for the total number of synthetically tractable organic molecules containing carbon, hydrogen, nitrogen, oxygen, sulfur, and halogens limited to molecular weights below 500 g/mol vary between 10^{20} and 10^{60} molecules [86].

Two common approaches are used when applying DOS in medicinal chemistry. The first involves the expansion of a screening library so that a thorough study of functional groups, configurations, and conformations can be achieved during the primary HTS screening stage, as in the case of the β -cell apoptosis inhibitors [89]. This method offers an advanced starting point for the medicinal chemistry stage of a project, because the compounds included are so diverse yet methodical in stereochemistry and scaffold evaluation. The second approach involves the application of DOS to hit-to-lead development. Once a hit from a screen is identified, DOS can be used to synthesize a variety of structures in a targeted library rapidly to map out SAR information in a short amount of time, as demonstrated in the case of the Hsp70 inhibitors [95].

1. Inhibitors of Cytokine-Induced β -cell Apoptosis Discovered Via HTS

Type-1 diabetes is an autoimmune disease in which pancreatic insulin-producing β -cells undergo an increased rate of apoptosis due to an overproduction of cytokines such as IL-1 β , IFN- γ , and TNF- α [87]. These cytokines activate transcription factors such as NF κ B and STAT1, triggering the apoptotic pathway. An HTS screening campaign measuring cellular ATP levels as an indication of cell viability in a rat β -cell line treated with pro-inflammatory cytokines was used to find suppressors of this pathway [88]. The libraries tested in this HTS screen included several DOS libraries, among which one library was found to be particularly active. This library of fused medium-sized bicycles contained 6,488 compounds that were synthesized from stereochemically pure starting material (Figure 11.17) [89]. Starting with a chiral pool of desired amino alcohols and γ -amino acids, peptide coupling and reduction of the resulting amide were used to combine the building blocks. Acylation of the secondary amine followed by S_NAr cyclization provided the 8-membered lactam core. This synthesis utilized SynPhase solid support lanterns attached to a primary alcohol for ease of purification and handling. Capping of the aniline nitrogen by reaction with twenty-seven different building blocks, including sulfonyl chlorides, isocyanates, acyl chlorides, and aldehydes, provided a diverse set of intermediates (Figure 11.17, blue R¹). Subsequent deprotection of the secondary amine and conversion to sulfonamides, ureas, or tertiary amines yielded a diverse collection of analogs as a set of eight stereoisomers (Figure 11.17, red R²).

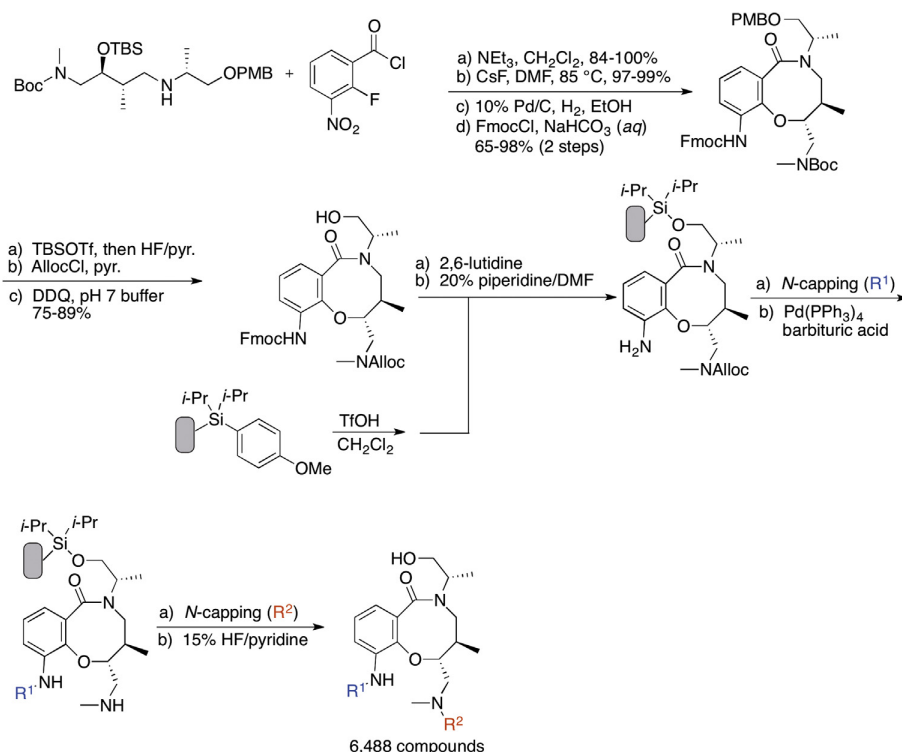


FIGURE 11.17 DOS library synthesis of 6-8 fused rings that were found to be hits in a β -cell apoptosis inhibitors screen. Only the *S,R,R* enantiomer is shown but each possible stereoisomer was synthesized. This figure was adapted from Ref. [89].

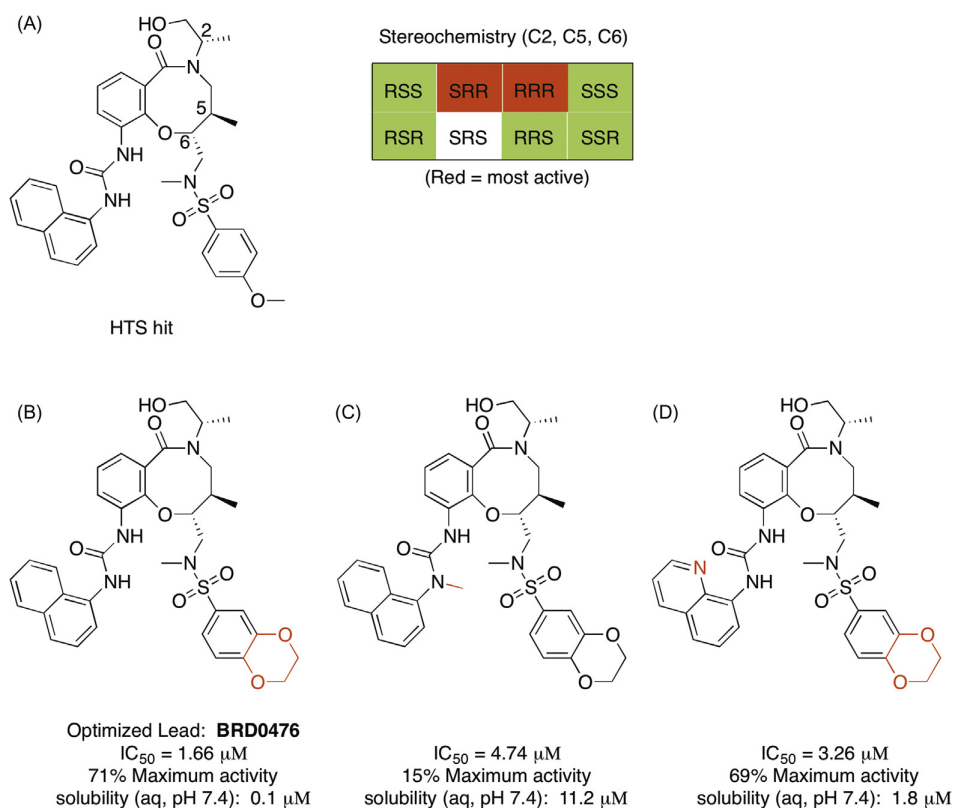


FIGURE 11.18 Structures of (A) an HTS hit and analogs with the incorporation of (B) a fused 1,4-dioxane, (C) a methyl urea, and (D) a quinoline, and their effects on potency and solubility [88,89].

The HTS screening hit is shown in Figure 11.18A. These data can be summarized in a heat map format with red boxes indicating the most active analogs and each 2×4 box showing all of the possible stereoisomers of a given analog. This heat map allows for a visualization of the activity and a graphical overview the SAR (structure-activity relationship) as well as SSAR (stereochemical structure-activity relationship) for all 6,488 analogs. The most potent compound in the original screen was the 2*S*,5*R*,6*R*-derivative with a naphthyl urea at R^1 and a *p*-methoxy phenyl sulfonamide at R^2 (Figure 11.18, A), exhibiting an EC_{50} of $4.89 \mu M$. A comparison to other hits showed that the configuration within the 8-membered ring (C5 and C6) had a significant contribution to the activity, while the exocyclic stereocenter (C2) was inconsequential. For example, the (2*S*,5*S*,6*R*)-diastereomer of the most potent hit displayed an activity of $<15\%$ in the inhibition of apoptosis (Figure 11.18). Additionally, the bulky naphthyl urea was necessary for activity, as several analogs with smaller phenyl rings were found to be less potent.

Based on the SAR and SSAR that could be gained from the HTS, analog BRD0476 (Figure 11.18, B) was found to be the most potent suppressor of β -cell apoptosis with an IC_{50} of $1.66 \mu M$, and it restored β -cell activity to 71 percent function. Mechanism of action studies of the optimized hit were challenging, due to the low aqueous solubility of the compound. Analog, including a derivative with a methyl group on the urea (Figure 11.18, C), were synthesized to modify the dihedral angle between the urea and naphthyl groups. While the methyl urea analog was more soluble, its activity was only 15 percent, implying that the bulky naphthyl group was in the desired position for activity when the unsubstituted urea was used. The incorporation of a quinoline in place of the naphthyl group solved this problem, in that it allowed for the necessary steric bulk but also added a H-bonding group to increase solubility (Figure 11.18, D). Mechanism of action studies of this compound demonstrated potent inhibition of the STAT1 signal transduction induced by IFN- γ [90].

The utility of DOS in HTS screening is clearly demonstrated by this example. The information that could be gained by having so many different analogs of a core structure with all stereoisomers included allowed for the rapid determination of functional groups that were important for activity. Follow-up chemistry could focus on a single diastereomer to allow for an advanced probe in a short period of time.

III. PRIMARY EXPLORATION OF STRUCTURE-ACTIVITY RELATIONSHIPS

2. Molecular Probes of Heat Shock Protein 70 (Hsp70) Derived from a Focused Library

The heat shock protein 70 (Hsp70) family forms a group of molecular chaperones that assist in the folding of nascent proteins. It also targets misfolded proteins for degradation and transport across biological membranes [91]. Hsp70s are antiapoptotic chaperones and are therefore implicated in cancer by acting at multiple points along the apoptotic pathway. Small molecules that modulate Hsp70 activity could find therapeutic applications as antitumor and antiviral agents as well as antibiotics, since viruses and bacteria also rely on the chaperones for survival. MAL3-101 [92] is an Hsp70 inhibitor that was prepared in the University of Pittsburgh Center for Chemical Methodologies and Library Development (UPCMLD) as part of a focused library based on the known Hsp70 modulator NSC 630668-R/1 [93] (Figure 11.19). MAL3-101 has fewer rotatable bonds than NSC 630668-R/1, but when the minimum energy conformations of the two compounds were superimposed via molecular modeling, the two structures overlaid well. More importantly, MAL3-101 selectively inhibited J-chaperone-stimulated Hsp70 ATPase activity in a concentration-dependent manner [92].

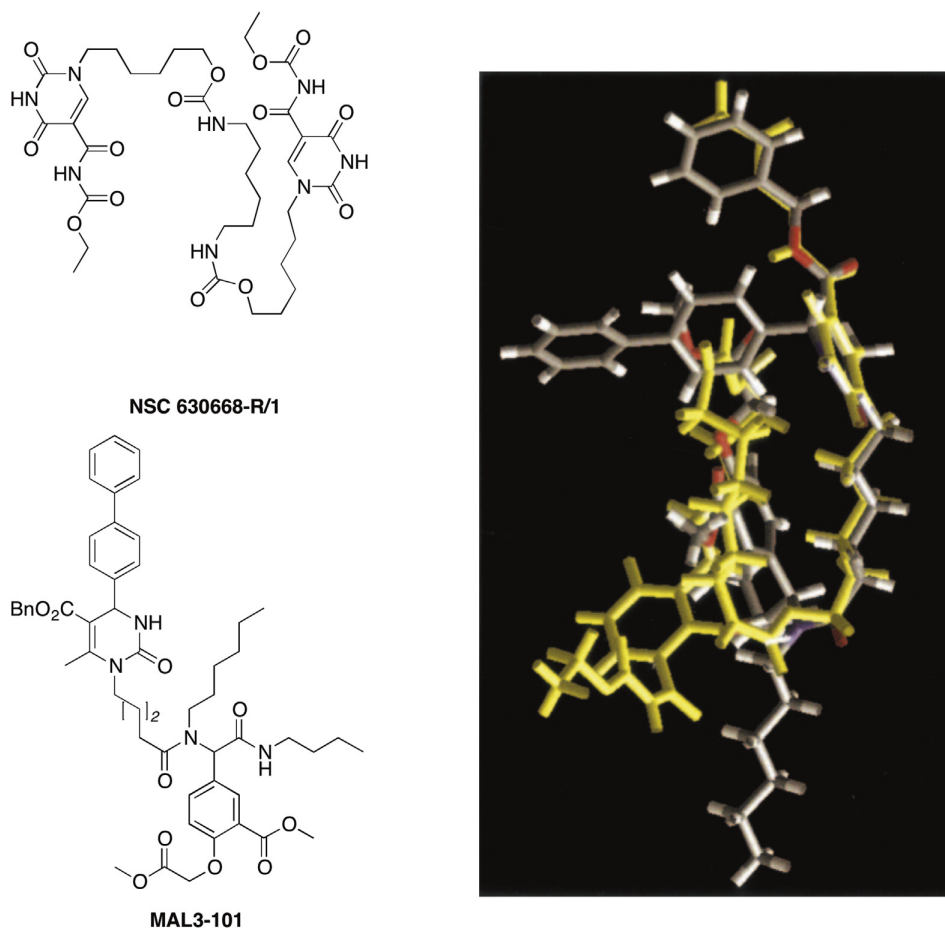


FIGURE 11.19 Superimposed structures of NSC 630668-R/1 and MAL3-101 after lowest energy conformer minimization. This research was originally published in the *Journal of Biological Chemistry*: Fewell SW, Smith CM, Lyon MA, Dumitrescu TP, Wipf P, Day BW, et al. Small molecule modulators of endogenous and co-chaperone-stimulated Hsp70 ATPase activity. *J Biol Chem* 2004;279:51131–40. Copyright the American Society for Biochemistry and Molecular Biology.

This library design relied on the use of two sequential multi-component reactions to synthesize efficiently analogs with seven points of diversification for screening purposes. The dihydropyrimidinones were synthesized in a one pot Biginelli reaction from the desired aldehyde, urea, and β -ketoester (Figure 11.20A), providing four points of diversification. An Ugi multi-component reaction then incorporated the appropriate amine, aldehyde, and alkyl cyanide building blocks, and contributed three additional points of diversification (Figure 11.20B).

III. PRIMARY EXPLORATION OF STRUCTURE-ACTIVITY RELATIONSHIPS

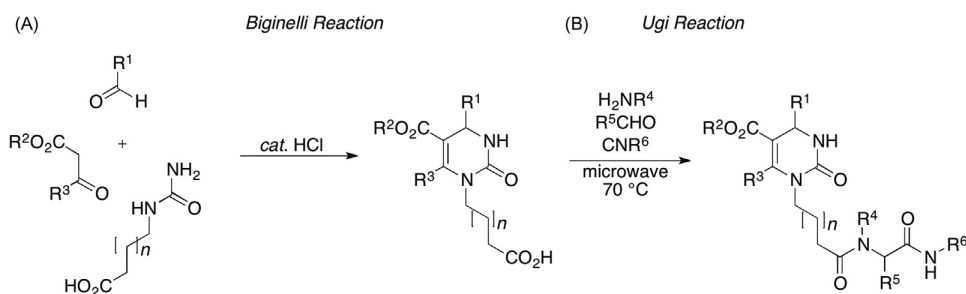


FIGURE 11.20 Diversity-oriented synthesis of the MAL3 library of Hsp70 probe molecules [94].

Following the successful discovery of MAL3-101, a second-generation library was synthesized using the same tandem Biginelli-Ugi reaction sequence [94]. In the first-generation library, it had been determined that a 5-carbon linker was optimal for Hsp70 inhibition (Figure 11.20, $n = 2$), and the importance of the biphenyl group in the R^1 position, the n -hexyl chain in the R^4 position, and the 5-formyl-2-methoxycarbonylmethoxybenzoic acid methyl ester at R^5 (Figure 11.20) were studied. This second-generation library led to the discovery of additional modulators of Hsp70 activity with diverging effects (Figure 11.21). Specifically, while MAL3-101 was an effective antimalarial, antiproliferative, and antitrypanosome agent, it had no effect on SV40. DMT3088 demonstrated improved antiproliferative activity over the first-generation analogs, and MAL2-11B showed SV40 activity (Figure 11.21), acting by inhibiting viral DNA synthesis. Interestingly, MAL2-213 had antimalarial activity but no antiproliferative activity [95]. The rapid access to conformationally more restricted NSC630668-R/1 analogs via a DOS strategy therefore allowed for the discovery of useful probes of Hsp70 with differing activities in cell proliferation, viral, and malaria assays.

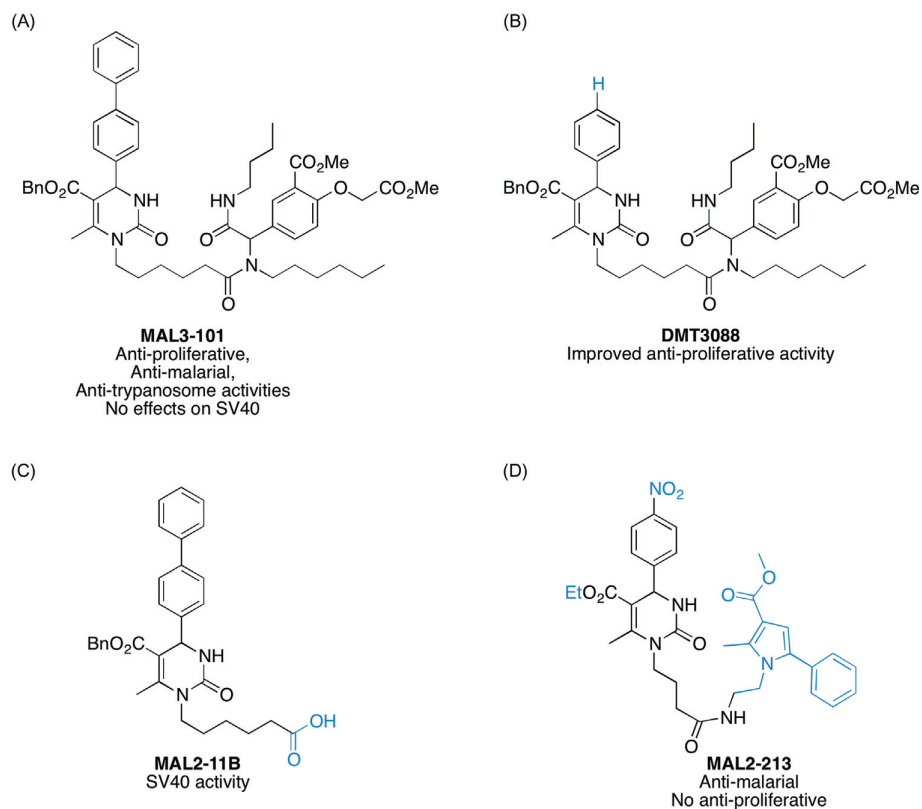


FIGURE 11.21 Differing activities of members of a focused Hsp70 library [95].

III. PRIMARY EXPLORATION OF STRUCTURE-ACTIVITY RELATIONSHIPS

III. SUMMARY AND OUTLOOK

In biological processes, noncovalent interactions are orchestrated by complex thermodynamic principles. If a lead compound has been identified, one strategy for further optimization is to introduce structural elements that reduce the conformational flexibility, and, if possible, pre-organize the ligand in a conformation complementary to the bioreceptor. As a consequence, the energy penalty (entropic factor) associated with the binding can be diminished with respect to the flexible parent compound. This thermodynamic advantage can be leveraged into an improved value of the affinity constant. This valuable medicinal chemistry strategy based on thermodynamic considerations remains rather empirical in nature but has been successful, mainly for the optimization of preliminary pharmacophore models and the test of pharmacophore working hypotheses. Furthermore, selected structural modifications of the ligand scaffold may improve both potency and selectivity. However, the rigidification of flexible ligands without loss of potency is challenging, and sophisticated chemistry is needed to achieve the syntheses of constrained compounds with complex architectures. In this respect, the use of chemistry tools such as annulation of substituents and DOS will continue to have an important impact.

References

- [1] Jencks WP. On the attribution and additivity of binding energies. *Proc Natl Acad Sci* 1981;78:4046–50.
- [2] Raffa RB, Porreca F. Thermodynamic analysis of the drug-receptor interaction. *Life Sci* 1989;44:245–58.
- [3] Andrews PR, Craik DJ, Martin JL. Functional group contributions to drug-receptor interactions. *J Med Chem* 1984;27:1648–57.
- [4] Page MI. Entropy, binding energy, and enzymic catalysis. *Angew Chem Int Ed Engl* 1977;16:449–59.
- [5] Bardi JS, Luque I, Freire E. Structure-based thermodynamic analysis of HIV-1 protease inhibitors. *Biochemistry* 1997;36:6588–96.
- [6] Velazquez-Campoy A, Todd MJ, Freire E. HIV-1 protease inhibitors: enthalpic versus entropic optimization of the binding affinity. *Biochemistry* 2000;39:2201–7.
- [7] Searle MS, Williams DH. The cost of conformational order: entropy changes in molecular associations. *J Am Chem Soc* 1992;114:10690–7.
- [8] Williams DH, Westwell MS. Aspects of weak interactions. *Chem Soc Rev* 1998;27:57–64.
- [9] Williams DH, Stephens E, O'Brien DP, Zhou M. Understanding noncovalent interactions: Ligand binding energy and catalytic efficiency from ligand-induced reductions in motion within receptors and enzymes. *Angew Chem Int Ed* 2004;43:6596–616.
- [10] Böhm H-J. The development of a simple empirical scoring function to estimate the binding constant for a protein-ligand complex of known three-dimensional structure. *J Comput Aided Mol Des* 1994;8:243–56.
- [11] Dunitz JD. Win some, lose some: enthalpy-entropy compensation in weak intermolecular interactions. *Chem Biol* 1995;2:709–12.
- [12] Gilli P, Ferretti V, Gilli G, Borea PA. Enthalpy-entropy compensation in drug-receptor binding. *J Phys Chem* 1994;98:1515–18.
- [13] Velazquez-Campoy A, Luque I, Freire E. The application of thermodynamic methods in drug design. *Thermochim Acta* 2001;380:217–27.
- [14] Weiland GA, Minneman KP, Molinoff PB. Fundamental difference between the molecular interactions of agonists and antagonists with the beta-adrenergic receptor. *Nature* 1979;281:114–17.
- [15] Miklavc A, Kocjan D, Mavri J, Koller J, Hadzi D. On the fundamental difference in the thermodynamics of agonist and antagonist interactions with β -adrenergic receptors and the mechanism of entropy-driven binding. *Biochem Pharmacol* 1990;40:663–9.
- [16] Borea PA, Dalpiaz A, Varani K, Guerra L, Gilli G. Binding thermodynamics of adenosine A2a receptor ligands. *Biochem Pharmacol* 1995;49:461–9.
- [17] Davis AM, Teague SJ. Hydrogen bonding, hydrophobic interactions, and failure of the rigid receptor hypothesis. *Angew Chem Int Ed* 1999;38:736–49.
- [18] Hruby VJ, Li G, Haskell-Luevano C, Shenderovich M. Design of peptides, proteins, and peptidomimetics in chi space. *Peptide Sci* 1997;43:219–66.
- [19] Gardiner J, Abell AD. Synthesis and solid-state conformation of phenylalanine mimetics constrained in a proline-like conformation. *Org Biomol Chem* 2004;2:2365–70.
- [20] Gibson SE, Jones JO, McCague R, Tozer MJ, Whitcombe NJ. A bromoarene based approach to phenylalanine analogues Hic and Nic. *Synlett* 1999;954–6.
- [21] Hanessian S, Bernstein N, Yang R-Y, Maguire R. Asymmetric synthesis of 1-azetidine-2-carboxylic acid and 3-substituted congener—conformationally constrained analogs of phenylalanine, naphthylalanine, and leucine. *Bioorg Med Chem Lett* 1999;9:1437–42.
- [22] Cativiela C, Lasa M, López P. Synthesis of enantiomerically pure cis- and trans-cyclopentane analogues of phenylalanine. *Tetrahedron Asymmetry* 2005;16:2613–23.
- [23] Pujadas G, Vaque M, Ardevol A, Blade C, Salvado MJ, Blay M, et al. Protein–ligand docking: a review of recent advances and future perspectives. *Curr Pharm Anal* 2008;4:1–19.
- [24] Kim R, Skolnick J. Assessment of programs for ligand binding affinity prediction. *J Comput Chem* 2008;29:1316–31.
- [25] Gao C, Park M-S, Stern HA. Accounting for ligand conformational restriction in calculations of protein-ligand binding affinities. *Biophys J* 2010;98:901–10.
- [26] Nicklaus MC, Wang S, Driscoll JS, Milne GWA. Conformational changes of small molecules binding to proteins. *Bioorg Med Chem* 1995;3:411–28.
- [27] Veber DF, Johnson SR, Cheng H-Y, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. *J Med Chem* 2002;45:2615–23.
- [28] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 1997;23:3–25.

- [29] Varma MVS, Obach RS, Rotter C, Miller HR, Chang G, Steyn SJ, et al. Physicochemical space for optimum oral bioavailability: Contribution of human intestinal absorption and first-pass elimination. *J Med Chem* 2010;53:1098–108.
- [30] Zimmerman DM, Leander JD, Cantrell BE, Reel JK, Snoddy J, Mendelsohn LG, et al. Structure-activity relationships of trans-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine antagonists for μ - and κ -opioid receptors. *J Med Chem* 1993;36:2833–41.
- [31] Zimmerman DM, Nickander R, Horng JS, Wong DT. New structural concepts for narcotic antagonists defined in a 4-phenylpiperidine series. *Nature* 1978;275:332–4.
- [32] Le Bourdonnec B, Goodman AJ, Michaut M, Ye H-F, Graczyk TM, Belanger S, et al. Elucidation of the bioactive conformation of the n-substituted trans-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of μ -opioid receptor antagonists. *J Med Chem* 2006;49:7278–89.
- [33] Manglik A, Kruse AC, Kobilka TS, Thian FS, Mathiesen JM, Sunahara RK, et al. Crystal structure of the μ -opioid receptor bound to a morphinan antagonist. *Nature* 2012;485:321–6.
- [34] Ōki M. Recent advances in atropisomerism N.p. In: Allinger NL, Eliel EL, Wilen SH, editors. *Topics in stereochemistry*, vol. 14. John Wiley & Sons, Inc; 1983. p. 1–81.
- [35] Christie GH, Kenner J. LXXI: the molecular configurations of polynuclear aromatic compounds. Part I. The resolution of γ -6 : 6'-dinitro- and 4 : 6 : 4' : 6'-tetranitro-diphenic acids into optically active components. *J Chem Soc Trans* 1922;121:614–20.
- [36] LaPlante SR, Edwards PJ, Fader LD, Jakalian A, Hucke O. Revealing atropisomer axial chirality in drug discovery. *Chem Med Chem* 2011;6:505–13.
- [37] LaPlante SR, Fader LD, Fandrick KR, Fandrick DR, Hucke O, Kemper R, et al. Assessing atropisomer axial chirality in drug discovery and development: natural products and drugs. *Chirality* 2011;25:265–74.
- [38] Zask A, Murphy J, Ellestad GA. Biological stereoselectivity of atropisomeric natural products and drugs. *Chirality* 2013;25:265–74.
- [39] Food and Drug Administration. Policy statement on the development of new stereoisomeric drugs. Available from: <<http://www.fda.gov/drugs/DrugInformation/Information/Guidances/ucm122883.htm>>; 2011 [accessed 13.10.20].
- [40] Olshansky SJ, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, et al. A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med* 2005;352:1138–45.
- [41] Ohki-Hamazaki H, Watase K, Yamamoto K, Ogura H, Yamano M, Yamada K, et al. Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity. *Nature* 1997;390:165–9.
- [42] Ladenheim EE, Hamilton NL, Behles RR, Bi S, Hampton LL, Battey JF, et al. Factors contributing to obesity in bombesin receptor subtype-3-deficient mice. *Endocrinology* 2008;149:971–8.
- [43] Guan X-M, Chen H, Dobbelaar PH, Dong Y, Fong TM, Gagen K, et al. Regulation of energy homeostasis by bombesin receptor subtype-3: selective receptor agonists for the treatment of obesity. *Cell Metab* 2010;11:101–12.
- [44] Welch CJ, Gong X, Schafer W, Chobanian H, Lin L, Biba M, et al. Factors influencing the interconversion of a new class of dibenzodiazepine sulfonamide atropisomers. *Chirality* 2009;21:E105–9.
- [45] Liu P, Lanza TJ, Chioda M, Jones C, Chobanian HR, Guo Y, et al. Discovery of benzodiazepine sulfonamide-based bombesin receptor subtype 3 agonists their unusual chirality. *ACS Med Chem Lett* 2011;2:933–7.
- [46] Chobanian HR, Guo Y, Liu P, Chioda M, Lanza TJ, Chang L, et al. Discovery of MK-7725, a potent, selective bombesin receptor subtype-3 agonist for the treatment of obesity. *ACS Med Chem Lett* 2012;3:252–6.
- [47] Sugane T, Tobe T, Hamaguchi W, Shimada I, Maeno K, Miyata J, et al. Atropisomeric 4-phenyl-4H-1,2,4-triazoles as selective glycine transporter 1 inhibitors. *J Med Chem* 2013;56:5744–56.
- [48] Bröer S, Cavanaugh JA, Rasko JEJ. Neutral amino acid transport in epithelial cells and its malfunction in Hartnup disorder. *Biochem Soc Trans* 2005;33:233–6.
- [49] Sur C, Kinney GG. Glycine transporter 1 inhibitors and modulation of NMDA receptor-mediated excitatory neurotransmission. *Curr Drug Targets* 2007;8:643–9.
- [50] Danysz W, Parsons CG. Glycine and N-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. *Pharmacol Rev* 1998;50:597–664.
- [51] Sugane T, Tobe T, Hamaguchi W, Shimada I, Maeno K, Miyata J, et al. Synthesis and biological evaluation of 3-biphenyl-4-yl-4-phenyl-4H-1,2,4-triazoles as novel glycine transporter 1 inhibitors. *J Med Chem* 2011;54:387–91.
- [52] Gause GF, Brazhnikova MG. Gramicidin S. and its use in the treatment of infected wounds. *Nature* 1944;154:703.
- [53] Stern A, Gibbons WA, Craig LC. A conformational analysis of gramicidin S-A by nuclear magnetic resonance. *Proc Natl Acad Sci* 1968;61:734–41.
- [54] Yamada K, Unno M, Kobayashi K, Oku H, Yamamura H, Araki S, et al. Stereochemistry of protected ornithine side chains of gramicidin S derivatives: X-ray crystal structure of the bis-Boc-tetra-N-methyl derivative of gramicidin S. *J Am Chem Soc* 2002;124:12684–8.
- [55] Xiao J, Weisblum B, Wipf P. Electrostatic versus steric effects in peptidomimicry: synthesis and secondary structure analysis of gramicidin S analogues with (E)-alkene peptide isosteres. *J Am Chem Soc* 2005;127:5742–3.
- [56] Grotenbreg GM, Kronemeijer M, Timmer MSM, El Oualid F, van Well RM, Verdoes M, et al. A practical synthesis of gramicidin S and sugar amino acid containing analogues. *J Org Chem* 2004;69:7851–9.
- [57] Wipf P, Xiao J, Stephenson CRJ. Peptide-like molecules (plms): a journey from peptide bond isosteres to gramicidin S mimetics and mitochondrial targeting agents. *Chimia* 2009;63:764–75.
- [58] Skoda EM, Davis GC, Wipf P. Allylic amines as key building blocks in the synthesis of (E)-alkene peptide isosteres. *Org Process Res Develop* 2012;16:26–34.
- [59] Frantz M-C, Pierce JG, Pierce JM, Kangying L, Qingwei W, Johnson M, et al. Large-scale asymmetric synthesis of the bioprotective agent JP4-039 and analogs. *Org Lett* 2011;13:2318–21.
- [60] Xiao J, Weisblum B, Wipf P. Trisubstituted (E)-alkene dipeptide isosteres as β -turn promoters in the gramicidin S cyclodecapeptide scaffold. *Org Lett* 2006;8:4731–4.
- [61] Fink MP, Macias CA, Xiao J, Tyurina YY, Jiang J, Belikova N, et al. Hemigramicidin-TEMPO conjugates: novel mitochondria-targeted anti-oxidants. *Biochem Pharmacol* 2007;74:801–9.

III. PRIMARY EXPLORATION OF STRUCTURE-ACTIVITY RELATIONSHIPS

- [62] Tyurina YY, Tyurin VA, Kaynar AM, Kapralova VI, Wasserloos K, Li J, et al. Oxidative lipidomics of hyperoxic acute lung injury: mass spectrometric characterization of cardiolipin and phosphatidylserine peroxidation. *Am J Physiol* 2010;299:L73–85.
- [63] Ji J, Kline AE, Amoscato A, Samhan-Arias AK, Sparvero LJ, Tyurin VA, et al. Lipidomics identifies cardiolipin oxidation as a mitochondrial target for redox therapy of brain injury. *Nat Neurosci* 2012;15:1407–13.
- [64] Nesbitt KM, Jaquins-Gerstl A, Skoda EM, Wipf P, Michael AC. Pharmacological mitigation of tissue damage during brain microdialysis. *Analyt Chem* 2013;85:8173–9.
- [65] Xun Z, Rivera-Sánchez S, Ayala-Peña S, Lim J, Budworth H, Skoda EM, et al. Targeting of XJB-5-131 to mitochondria suppresses oxidative DNA damage and motor decline in a mouse model of Huntington's disease. *Cell Rep* 2012;2:1137–42.
- [66] Frantz M-C, Skoda EM, Sacher JR, Epperly MW, Goff JP, Greenberger JS, et al. Synthesis of analogs of the radiation mitigator JP4-039 and visualization of BODIPY derivatives in mitochondria. *Org Biomol Chem* 2013;11:4147–53.
- [67] Barreiro EJ, Kümmerle AE, Fraga CAM. The methylation effect in medicinal chemistry. *Chem Rev* 2011;111:5215–46.
- [68] Leung CS, Leung SSF, Tirado-Rives J, Jorgensen WL. Methyl effects on protein–ligand binding. *J Med Chem* 2012;55:4489–500.
- [69] Némethy G. Hydrophobe wechselwirkungen [Hydrophobic interactions]. *Angew Chem* 1967;79:260–71 [in German].
- [70] Southall NT, Dill KA, Haymet ADJ. A view of the hydrophobic effect. *J Phys Chem B* 2001;106:521–33.
- [71] Schönherr H, Cernak T. Profound methyl effects in drug discovery and a call for new C–H methylation reactions. *Angew Chem Int Ed* 2013;52:12256–67.
- [72] Quancard J, Bollbuck B, Janser P, Angst D, Berst F, Buehlmayr P, et al. A potent and selective S1P1 antagonist with efficacy in experimental autoimmune encephalomyelitis. *Chem Biol* 2012;19:1142–51.
- [73] Goodman AJ, Le Bourdonnec B, Dolle RE. Mu opioid receptor antagonists: recent developments. *Chem Med Chem* 2007;2:1552–70.
- [74] Jenkins GM, Frohman MA. Phospholipase D: a lipid centric review. *Cell Mol Life Sci* 2005;62:2305–16.
- [75] Elvers M, Stegner D, Hagedorn I, Kleinschnitz C, Braun A, Kuijpers MEJ, et al. Impaired α IIb β 3 integrin activation and shear-dependent thrombus formation in mice lacking phospholipase D1. *Sci Signal* 2010;3:1–10.
- [76] Oliveira TG, Chan RB, Tian H, Laredo M, Shui G, Staniszewski A, et al. Phospholipase D2 ablation ameliorates Alzheimer's disease-linked synaptic dysfunction and cognitive deficits. *J Neurosci* 2010;30:16419–28.
- [77] O'Reilly MC, Scott SA, Brown KA, Oguin TH, Thomas PG, Daniels JS, et al. Development of dual PLD1/2 and PLD 2 selective inhibitors from a common 1,3,8-triazaspiro[4.5]decane core: discovery of ML298 and ML299 that decrease invasive migration in U87-MG glioblastoma cells. *J Med Chem* 2013;56:2695–9.
- [78] Czekaj M, Klein SI, Guertin KR, Gardner CJ, Zulli AL, Pauls HW, et al. Optimization of the β -aminoester class of factor Xa inhibitors. Part 1: P4 and side-chain modifications for improved in vitro potency. *Bioorg Med Chem Lett* 2002;12:1667–70.
- [79] Schärfer C, Schulz-Gasch T, Ehrlich H-C, Guba W, Rarey M, Stahl M. Torsion angle preferences in druglike chemical space: a comprehensive guide. *J Med Chem* 2013;56:2016–28.
- [80] Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* 2006;7:606–19.
- [81] Fontana L, Partridge L, Longo VD. Extending healthy life span—from yeast to humans. *Science* 2010;328:321–6.
- [82] Liu KKC, Huang X, Bagrodia S, Chen JH, Greasley S, Cheng H, et al. Quinazolines with intra-molecular hydrogen bonding scaffold (iMHBS) as PI3K/mTOR dual inhibitors. *Bioorg Med Chem Lett* 2011;21:1270–4.
- [83] LaPlante SR, Gillard JR, Jakalian A, Aubry N, Coulombe R, Brochu C, et al. Importance of ligand bioactive conformation in the discovery of potent indole-diamide inhibitors of the Hepatitis C virus NS5B. *J Am Chem Soc* 2010;132:15204–12.
- [84] Spring DR. Diversity-oriented synthesis: a challenge for synthetic chemists. *Org Biomol Chem* 2003;1:3867–70.
- [85] Virshup AM, Contreras-García J, Wipf P, Yang W, Beratan DN. Stochastic voyages into uncharted chemical space produce a representative library of all possible drug-like compounds. *J Am Chem Soc* 2013;135:7296–303.
- [86] Fink T, Bruggesser H, Raymond J-L. Virtual exploration of the small-molecule chemical universe below 160 daltons. *Angew Chem Int Ed* 2005;44:1504–8.
- [87] Cnop M, Welsh N, Jonas J-C, Jörns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic β -cell death in type 1 and type 2 diabetes: many differences, few similarities. *Diabetes* 2005;54:S97–107.
- [88] Chou DH-C, Bodycombe NE, Carrinski HA, Clemons PA, Schreiber SL, et al. Small-molecule suppressors of cytokine-induced β -cell apoptosis. *ACS Chem Biol* 2010;5:729–34.
- [89] Chou DH-C, Duvall JR, Gerard B, Liu H, Pandya BA, Suh B-C, et al. Synthesis of a novel suppressor of β -cell apoptosis via diversity-oriented synthesis. *ACS Med Chem Lett* 2011;2:698–702.
- [90] Scully SS, Tang AJ, Lundh M, Mosher CM, Perkins KM, Wagner BK. Small-molecule inhibitors of cytokine-mediated STAT1 signal transduction in β -cells with improved aqueous solubility. *J Med Chem* 2013;56:4125–9.
- [91] Hartl FU. Molecular chaperones in cellular protein folding. *Nature* 1996;381:571–80.
- [92] Fewell SW, Smith CM, Lyon MA, Dumitrescu TP, Wipf P, Day BW, et al. Small molecule modulators of endogenous and co-chaperone-stimulated Hsp70 ATPase activity. *J Biol Chem* 2004;279:51131–40.
- [93] Fewell SW, Day BW, Brodsky JL. Identification of an inhibitor of Hsc70-mediated protein translocation and ATP hydrolysis. *J Biol Chem* 2001;276:910–14.
- [94] Werner S, Turner DM, Lyon MA, Huryn DM, Wipf P. A focused library of tetrahydropyrimidinone amides via a tandem Biginelli-Ugi multi-component process. *Synlett* 2006;14:2334–8.
- [95] Huryn DM, Brodsky JL, Brummond KM, Chambers PG, Eyer B, Ireland AW, et al. Chemical methodology as a source of small-molecule checkpoint inhibitors and heat shock protein 70 (Hsp70) modulators. *Proc Natl Acad Sci* 2011;108:6757–62.

III. PRIMARY EXPLORATION OF STRUCTURE-ACTIVITY RELATIONSHIPS