

POLYPHARMACOLOGY

STRATEGIES FOR MULTI-TARGET DRUG DISCOVERY



EDITED BY

JENS-UWE PETERS

WILEY

Polypharmacology

Polypharmacology

Strategies for Multi-Target Drug Discovery

Edited by
Jens-Uwe Peters

WILEY

Copyright © 2025 by John Wiley & Sons, Inc. All rights reserved, including rights for text and data mining and training of artificial technologies or similar technologies.

Published by John Wiley & Sons, Inc., Hoboken, New Jersey.
Published simultaneously in Canada.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning, or otherwise, except as permitted under Section 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4470, or on the web at www.copyright.com. Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, (201) 748-6011, fax (201) 748-6008, or online at <http://www.wiley.com/go/permission>

Trademarks: Wiley and the Wiley logo are trademarks or registered trademarks of John Wiley & Sons, Inc. and/or its affiliates in the United States and other countries and may not be used without written permission. All other trademarks are the property of their respective owners. John Wiley & Sons, Inc. is not associated with any product or vendor mentioned in this book.

Limit of Liability/Disclaimer of Warranty: While the publisher and author have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives or written sales materials. The advice and strategies contained herein may not be suitable for your situation. You should consult with a professional where appropriate. Further, readers should be aware that websites listed in this work may have changed or disappeared between when this work was written and when it is read. Neither the publisher nor authors shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

For general information on our other products and services or for technical support, please contact our Customer Care Department within the United States at (800) 762-2974, outside the United States at (317) 572-3993 or fax (317) 572-4002.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic formats. For more information about Wiley products, visit our web site at www.wiley.com.

Library of Congress Cataloging-in-Publication Data applied for:

Hardback ISBN: 9781394182831

Cover Design: Wiley
Cover Image: Courtesy of Balaguru Ravikumar

Set in 9.5/12.5pt STIXTwoText by Straive, Chennai, India

Contents

List of Contributors xvii

Preface xxiii

1 Introduction 1

Jürgen Bajorath

- 1.1 Origins 1
- 1.2 Pros and Cons 1
- 1.3 Discovery and Design 2
- 1.4 Structural Data 2
- 1.5 Activity Data 3
- 1.6 Drug Target Estimates 4
- 1.7 Explainable Machine Learning 5
- 1.8 Conclusion 6
- References 6

Part A Polypharmacology as a Safety Concern in Drug Discovery 9

2 The Safety Relevance and Interpretation of Compound Off-target Interactions 11

Eric A.G. Blomme, Jonathon R. Green, Prathap Kumar S. Mahalingaiah, Terry R. Van Vleet, and Andy Vo

- 2.1 Introduction 11
- 2.2 Assessing Off-Target Interactions of Small Molecules 12
- 2.3 Interpretation of Data from Secondary Pharmacology Assays 13
- 2.4 Off-Target Interactions of Biologics: Polyreactivity and Polyspecificity 14
- 2.5 Case Study Examples 16
 - 2.5.1 COX-2 Inhibition 16
 - 2.5.2 Acetylcholinesterase/Neuropathy Target Esterase 17
 - 2.5.3 VEGF Inhibition 17
- 2.6 Physicochemical Properties 18
- 2.7 *In Silico* Methods to Predict Off-Target Interactions 19
- 2.8 Predicting Antibody Specificity 19
- References 21

3 Off-target Activity and Adverse Drug Reactions 25

Dimitar Yonchev

- 3.1 Personal Perspective 25
- 3.2 Introduction 25
- 3.3 Secondary Pharmacology and Adverse Drug Reactions 26
 - 3.3.1 Important Considerations for Data Analysis 26
 - 3.3.2 Assessing the Clinical Relevance of Drug–Target Associations 27

3.3.3	Systematic Evaluation of Target–ADR Relationships	28
3.4	A Practical Perspective	31
	Acknowledgments	33
	References	34
4	Off-Target Screening Strategies	37
	<i>Sonia Roberts and Helen L. Lightfoot</i>	
4.1	Introduction	37
4.2	Small Molecules	37
4.3	Proteolysis-Targeting Chimeras (PROTACs)	39
4.4	Small Molecules Targeting RNA (smRNA)	41
4.5	Antisense Oligonucleotides	43
4.6	Large Molecules	43
4.7	Regulatory Aspects	44
4.8	Future Outlook	45
	Acknowledgments	45
	Addendum	45
	References	45
5	Molecular Properties and Structural Motifs Related to Pharmacological Promiscuity	49
	<i>Jens-Uwe Peters</i>	
5.1	Introduction	49
5.2	Basicity and Protonation State	49
5.2.1	Mitigating the Promiscuity of Basic Compounds	50
5.2.1.1	Reduction of Basicity below a [b]pK _a of 6	51
5.2.1.2	Creation of a Zwitterion	51
5.2.1.3	Reduction of the Number of Aromatic Rings to 1 or 0	51
5.2.1.4	Replacement of a Tertiary or Secondary Amine by a Primary Amine	51
5.2.2	Should We Avoid Basic Amines?	52
5.3	Lipophilicity	52
5.4	Molecular Weight	54
5.5	Other Parameters	54
5.6	Structural Motifs	54
5.6.1	Aminergic Motifs	54
5.6.2	Kinase Motifs	54
5.6.3	Other Motifs	54
5.7	Conclusion	56
	References	57
6	Kinase Liabilities in Early Drug Discovery	61
	<i>Stephan Kirchner</i>	
6.1	Introduction	61
6.2	Protein Kinases and Inhibitor Binding Sites	61
6.3	Kinase-regulated Cardiac Functions and Potential Consequences of Inhibition	64
6.4	Core Kinases Driving the Cell Division Cycle and Consequences of Interference	64
6.5	Cell Cycle Checkpoints Controlling Cell Division	69
6.6	Selectivity Profiling of Kinase Inhibition	71
	References	72
7	Activity at Cardiovascular Ion Channels	77
	<i>Ian M. Bell and Armando A. Lagrutta</i>	
7.1	Introduction	77

7.2	Screening Methods	79
7.3	Structural Insights into the Interaction Between Drugs and CV Ion Channels	80
7.4	Medicinal Chemistry Approaches	85
7.5	Conclusion	90
	References	91

Part B Polypharmacology as an Opportunity in Different Disease Areas 97

8	Toward Mechanism-based Therapies and Network Pharmacology	99
	<i>Cristian Nogales, Zina Piper, Zeinab Mamdouh, and Mayra Pacheco Pachado</i>	
8.1	A Crisis in the Pharmaceutical Industry	99
8.2	Disease Modules as Targets for Precision Medicine	99
8.3	Mechanism-based Therapies and Network Pharmacology	101
8.4	Implementing Mechanism-based Therapies	103
8.4.1	Cancer	103
8.4.2	Inborn Errors of Metabolism	103
8.4.3	Human Immunodeficiency Virus (HIV)	104
8.4.4	ROS/cGMP Cluster of Cardiovascular, Respiratory, Metabolic, and Neurological Disease Phenotypes	105
8.5	Summary and Conclusions	105
	References	106
9	Advancements in Rational Multi-Targeted Drug Discovery	109
	<i>Balaguru Ravikumar, Anna Cichon'ska, Navriti Sahni, Tero Aittokallio, and Rayees Rahman</i>	
9.1	Introduction	109
9.2	Cancer and the Existing Treatment Strategies	109
9.2.1	Overview of Cancer and its Complexity	109
9.2.2	Historical Perspective on Cancer Drug Discovery	110
9.2.3	Drug Resistance in Targeted Therapies	111
9.2.4	Rational Combination Therapies	111
9.2.5	Rational Targeted Polypharmacology	111
9.2.6	Protein Kinases and Small Molecule KIs	112
9.2.7	Review of FDA-Approved Multi-Targeted Inhibitors	113
9.3	Safety and Efficacy: A Double-Edged Sword	114
9.3.1	Approaches for Target Selection for MTDs	114
9.3.2	Anti-Targets for MTD Design	115
9.4	Rational Design of MTDs	116
9.4.1	Rational Design of MTDs Through Medicinal Chemistry	116
9.4.2	Computational Methods to Design MTDs	117
9.4.3	AI Models and Their Role in MTDs	118
9.5	Perspective, Limitations, and Challenges	120
	References	120
10	Polypharmacology	127
	<i>Lynn L. Silver</i>	
10.1	Introduction	127
10.2	The Failure of Single-target-based Discovery of Antibiotics	127
10.3	Attempts at Purposeful Multitargeting	128
10.3.1	Mur Ligases and a Caveat	128
10.3.2	Protein Synthesis Inhibition by Ribosomal RNA Binders	129
10.3.3	tRNA Synthetases as Targets	129

10.3.4	New Topoisomerase Inhibitors	130
10.3.4.1	Gepotidacin	130
10.3.4.2	Zoliflodacin	131
10.3.4.3	Kibdelomycin	131
10.3.4.4	Albicidin	131
10.4	Cell Surface Targets and Macrocyclic Peptides (MCPs)	131
10.4.1	Relevant Permeability Properties of Bacteria	131
10.4.2	Cell Surface Targets	132
10.4.3	Polypharmacology and the Benefits of MCPs	132
10.4.4	Classic MCPs Targeting Cell Surfaces	132
10.4.4.1	Polymyxin	132
10.4.4.2	Vancomycin	132
10.4.5	New Gram-positive MCP Agents	133
10.4.5.1	Teixobactin	133
10.4.5.2	Clovibactin	133
10.4.5.3	Cilagicin	133
10.4.6	Gram-negative Inhibitors Targeting Cell Surfaces, Including OMPTAs	133
10.4.6.1	Darobactin	133
10.4.6.2	MRL-494	136
10.4.6.3	Murepavadin	136
10.4.6.4	Zosurabalpin	136
10.5	Conclusions	136
	References	136
11	Multi-Specific Binding Strategy	141
	<i>Yang Zhou, Shujing Xu, Dang Ding, Kai Tang, Xinyong Liu, Meehyein Kim, and Peng Zhan</i>	
11.1	Proteolysis Targeting Chimera (PROTAC)	142
11.2	Antibody Recruiting Molecules	147
11.3	Antibody-Drug Conjugates (ADCs)	150
11.4	Antiviral Drug Delivery Systems	151
11.4.1	Nucleic Acid Delivery Molecules	151
11.4.2	Cholesterol Conjugated Molecules	155
11.4.3	Human Serum Albumin Drug Loading	155
11.5	Ribonuclease Targeting Chimeras	155
11.6	Other Bifunctional Small Molecules	157
11.7	Summary and Outlook	159
	References	160
12	Polypharmacology for the Treatment of Major Depressive Disorder	165
	<i>Tiffany Schwasinger-Schmidt</i>	
12.1	Introduction	165
12.2	Multitargeted Antidepressants	166
12.2.1	Tricyclic Antidepressants	166
12.2.2	Monoamine Oxidase Inhibitors	167
12.2.3	Selective Serotonin Reuptake Inhibitors	167
12.2.4	Selective Serotonin and Norepinephrine Reuptake Inhibitors	168
12.2.5	Bupropion	169
12.2.6	Mirtazapine	169
12.2.7	Trazadone	169
12.2.8	Vilazodone	170
12.2.9	Vortioxetine	170

12.2.10	Triple Receptor Reuptake Agonists/Antagonists	170
12.3	Conclusions	170
	References	171
13	Multi-target Drugs to Treat Metabolic Diseases	175
	<i>Felix F. Lillich, Samaneh Goorani, Ewgenij Proschak, and John D. Imig</i>	
13.1	Introduction	175
13.2	Metabolic Diseases and Current Treatment Approaches	175
13.3	Strategies to Develop Multi-target Drugs for Metabolic Diseases	177
13.4	Approaches Involving Modulation of PPARs and Other Metabolically Relevant Nuclear Receptors	180
13.4.1	PPAR- γ /SUR	180
13.4.2	PPARs/FFAR1	180
13.4.3	PPAR- γ /GK	180
13.4.4	PPAR- γ /sEH	180
13.4.5	FXR/sEH	181
13.4.6	PPAR- γ or PPAR- α / γ /PTP1B	181
13.5	Approaches Involving Inhibition of DPP4	181
13.5.1	DPP4/ACE	182
13.5.2	DPP4/GPR119	182
13.5.3	DPP4/MCH-1R	182
13.6	Diverse Target Combinations for Polypharmacological Treatment of Metabolic Disorders	183
13.6.1	PTP1B/AMPK	183
13.6.2	SGLT/GP	184
13.7	Conclusion	184
	References	185
14	Overcoming the Challenges of Multi-Target-Directed Ligands for Alzheimer's Disease	193
	<i>Elisa Uliassi, Anna M. Pasieka, Eleonora Diamanti, and Maria Laura Bolognesi</i>	
14.1	Introduction	193
14.2	Target Identification: In the Search for New Target Pairs	193
14.3	PK Challenges in MTDL Optimization	195
14.4	Phenotypic Screening: In a Search for an Early Proof-of-Concept	197
14.5	Conclusions	199
	References	199
15	The Role of Polypharmacology in the History of Drug Discovery	203
	<i>Axel Helmstaedter</i>	
15.1	Introduction: Drug Discovery in the Twentieth Century	203
15.1.1	Medicinal Chemistry	203
15.1.2	The Evolution of the Receptor Concept	203
15.1.3	The Race for Receptor Specificity	204
15.2	Natural Products	205
15.3	Historical Drugs with Multiple Actions	206
15.3.1	Aspirin	206
15.3.2	Sulfonamides	206
15.3.3	Psychopharmaceuticals	207
15.3.4	Amantadine	208
15.3.5	Minoxidil/Finasteride	208
15.3.6	Sildenafil	209
15.4	From Serendipity to Concept: Repurposing and Polypharmacology	209
	References	210

Part C How to Discover Polypharmacological Drugs 213

16 Strategies for Multi-target Drug Discovery 215

Dayong Shi and Xiangqian Li

- 16.1 Introduction 215
- 16.2 Rational Design of Multitargeted Ligands 215
 - 16.2.1 Similar Pharmacophoric Elements 216
 - 16.2.1.1 HDAC and HMGR 216
 - 16.2.2 Similar Scaffold Structures 217
 - 16.2.2.1 FXR and sEH 217
 - 16.2.3 Targets Lacking Similar Ligands or Similar Endogenous Substrates 218
 - 16.2.3.1 AChE and GSK-3 β 218
 - 16.2.3.2 IN and RT 218
 - 16.3 Discussion and Conclusion 220
 - References 220

17 Predicting Polypharmacology with Web-Based Tools 223

Maedeh Darsaraee, Sacha Javor, and Jean-Louis Reymond

- 17.1 Introduction 223
- 17.2 PASS 223
 - 17.2.1 Data and Methodology 223
 - 17.2.2 Evaluation 225
- 17.3 SEA 226
 - 17.3.1 Data and Methodology 226
 - 17.3.2 Evaluation 226
- 17.4 Super-PRED 226
 - 17.4.1 Data and Methodology 226
 - 17.4.2 Evaluation 227
- 17.5 TargetHunter 227
 - 17.5.1 Data and Methodology 227
 - 17.5.2 Evaluation 227
- 17.6 SwissTargetPrediction 227
 - 17.6.1 Data and Methodology 227
 - 17.6.2 Evaluation 229
- 17.7 TargetNet 229
 - 17.7.1 Data and Methodology 229
 - 17.7.2 Evaluation 229
- 17.8 PPB 229
 - 17.8.1 Data and Methodology 229
 - 17.8.2 Evaluation 230
- 17.9 PPB2 230
 - 17.9.1 Data and Methodology 230
 - 17.9.2 Evaluation 230
- 17.10 Comparison of Different Web-Based Tools 231
- 17.11 Conclusion 233
- Acknowledgement 233
- References 233

18 Using Phenotypic Screening to Uncover the Full Potential of Polypharmacology 237

Arsenio Nueda

- 18.1 Introduction: Phenotypic Screening and Phenotypic Drug Discovery 237
- 18.2 Polypharmacology Discovered Using Phenotypic Screening 239

18.3	PDD Strategies to Discover Novel Polypharmacology	240
18.3.1	Disease Model	240
18.3.2	Library	240
18.3.3	Running a Phenotypic Screening and Selecting Hits	241
18.4	Optimizing Polypharmacology in Phenotypic Screening Hits	242
18.4.1	Hit Expansion and Hit Characterization	242
18.4.2	Optimizing Polypharmacology	242
18.5	Understanding the MoA from a PDD and Polypharmacology Perspectives	245
18.6	The Path to Virtual PDD-Derived Polypharmacology	246
18.7	Conclusions and Future Directions	246
	References	248
19	Phenotypic Polypharmacology Drug Discovery for CNS Applications	251
	<i>Alberto Ambesi-Impiombato, Lee McDermott, Alan Lars Pehrson, and Daniela Brunner</i>	
19.1	Introduction	251
19.2	BPDD Lessons from the History of Psychopharmacology	251
19.2.1	Antipsychotics and Antidepressants	252
19.2.2	Other Antidepressants	252
19.2.3	Mood Stabilizers	253
19.2.4	Anxiolytics and Sedatives	253
19.3	Current Trends in Psychopharmacology	253
19.3.1	Lessons from History	253
19.3.2	New Trends in Industry and Biotech	254
19.3.3	Novel Antipsychotics	254
19.4	A Machine Learning-Based System for Global Behavior Profiling for CNS Drug Discovery	255
19.4.1	Standardization of Screening Tools	255
19.4.2	The SmartCube System®	256
19.5	Modeling Chemical and Phenotypic Relationships of Compounds Screened in SmartCube®	257
19.5.1	Antidepressants	257
19.5.2	Psychedelics	257
19.6	Privileged Scaffolds and BPDD with SmartCube®	260
19.7	Ulotaront (SEP-363856) a BPDD Case Study	261
19.8	Conclusions	262
	References	263
	Appendix	266
20	Multi-target Peptides for the Treatment of Metabolic Diseases	269
	<i>Martin Bossart and Gerhard Hessler</i>	
20.1	Introduction	269
20.2	Glucagon-like Peptide-1 (GLP-1) Receptor Agonists	269
20.3	Unimolecular Multiagonists Based on Glucagon-like Peptide-1 (GLP-1) Following the One-pharmacophore Approach	270
20.4	GLP-1 Receptor/Glucagon Receptor Dual Agonists	272
20.5	Clinical Advanced GLP-1/GCGR Dual Agonists	275
20.6	GLP-1 Receptor/Glucose-dependent Insulinotropic Polypeptide (GIP) Receptor Dual Agonists	277
20.7	GLP-1 Receptor/Glucagon Receptor/GIP Receptor Triple Agonists	279
20.8	Further Unimolecular Multiagonists Based on Glucagon-like Peptide-1 (GLP-1) Following the One-pharmacophore Approach	280
20.9	Unimolecular Multiagonists Based on Glucagon-like Peptide-1 (GLP-1) Following the Two-pharmacophore Approach	282
20.10	Conclusion	284
	References	284

21 The SOSA Approach to Drug Discovery 289*Norbert Handler, Michal Poznik, and Helmut Buschmann*

- 21.1 Introduction 289
 - 21.1.1 Drug Selectivity and Unwanted or Desired Side Effects 289
- 21.2 Definition, Rational, and Concept of the SOSA Approach 290
 - 21.2.1 Multiple Ligands and Polypharmacology 291
 - 21.2.2 Safety and Bioavailability 291
- 21.3 Drugs in Other Drugs: Drug as Fragments 291
 - 21.3.1 Drug Repositioning and Drug Repurposing 292
- 21.4 Old Drugs 292
- 21.5 The SOSA Approach and Analog Design 292
- 21.6 Patentability and Interference Risk of the SOSA Approach 293
 - 21.6.1 Analogization, Optimization, and Isosterism 296
- 21.7 Case Studies and Examples 296
 - 21.7.1 Sulfonamides 296
 - 21.7.2 Morphine Analogs 296
 - 21.7.3 Warfarin 296
 - 21.7.4 Sildenafil (Viagra) 296
 - 21.7.5 Thalidomide Analogs 297
 - 21.7.6 Bupropion 298
 - 21.7.7 Chlorpromazine 298
 - 21.7.8 Chlorothiazide 298
 - 21.7.9 Propranolol 298
 - 21.7.10 Minaprine Analogs 299
 - 21.7.11 Viloxazine Analogs 299
 - 21.7.12 Methylation in the SOSA Strategy of Drug Design 299
 - 21.7.13 Discovery of New Antiplasmodial Compounds 301
 - 21.7.14 Drugs Acting on Central Nervous System (CNS) Targets as Leads for Non-CNS Targets 301
 - 21.7.15 Mexiletine Derivatives as Orally Bioavailable Inhibitors of Urokinase-type Plasminogen Activator 302
 - 21.7.16 Amiloride Analogs as Inhibitors of the Urokinase-type Plasminogen Activator (uPA) 304
 - 21.7.17 Flavonoids with an Oligopolysulfated Moiety: A New Class of Anticoagulant Agents 306
 - 21.7.18 Clioquinol 306
 - 21.7.19 From Antimalarial Drugs to Antifungals 307
 - 21.7.20 From Immunosuppressives to Antifungals 309
 - 21.7.21 From Antipsychotics to Antifungals 309
 - 21.7.22 Diclofenac as Inhibitor of the Transthyretin Amyloid Formation 310
 - 21.7.23 Pranlukast 311
 - 21.7.24 Loxapine 311
 - 21.7.25 Computer-assisted SOSA 311
 - 21.7.26 Cinalukast 311
 - 21.7.27 Talinolol 312
- 21.8 Conclusion 312
 - Credit 313
 - References 313

Part D Polypharmacology, Classic Case Studies and Recent Research 319**22 Dual Inhibitors of CDK4/6 for Treating Cancer 321***Peter L. Toogood*

- 22.1 Introduction 321
- 22.2 Selectivity Profile of Approved CDK4/6 Inhibitors 321

22.2.1	Assessing CDK4/6 Inhibitor Selectivity	323
22.3	Clinical Experience with CDK4/6 Inhibitors	325
22.3.1	Indications and Use for Approved CDK4/6 Inhibitors	325
22.3.2	Mechanisms of Drug Resistance to CDK4/6 Inhibitors	326
22.3.3	CDK4/6 Biomarkers	327
22.3.4	CDK4/6 Inhibitors in Combination Therapy	328
22.3.5	CDK4/6 Inhibitors and Immunotherapy	329
22.4	New Approaches and Agents for CDK4/6 Inhibition	330
22.4.1	Reversible Inhibitors	330
22.4.2	PROTACs	331
22.5	Conclusion	331
	Acknowledgment	332
	References	332
23	Tapentadol, a Clinically Proven Analgesic with Two Mechanisms	339
	<i>Thomas Christoph, Helmut Buschmann, Norbert Handler, and Michal Poznik</i>	
23.1	Introduction	339
23.2	The Discovery of Tapentadol – From Morphine and Tramadol to the Discovery of Tapentadol	339
23.3	Pharmacokinetics of Tapentadol	342
23.4	The Polymorphic Forms of Tapentadol Hydrochloride	343
23.5	Pharmaceutical Salts of Tapentadol	344
23.6	Synthesis Routes to Tapentadol Hydrochloride	354
23.7	The Pharmacological Profile of Tapentadol as a Multiple Ligand for the Treatment of Several Types of Pain	356
23.7.1	Preclinical Development	356
23.7.2	Efficacy	357
23.7.3	Adverse Effects	361
23.8	Summary	363
	References	363
24	Thalidomide – From a Banned Drug to Molecular Glues, PROTACs, and New Concepts in Drug Discovery	367
	<i>Junichi Yamamoto, Hiroshi Handa, and Yuki Yamaguchi</i>	
24.1	Introduction	367
24.2	Thalidomide History: From Tragedy to Therapeutic Revival	367
24.2.1	The Early Promise and Subsequent Disaster	367
24.2.2	Rediscovery and Therapeutic Applications	368
24.2.3	The Discovery of CRBN: Paving the Way for Targeted Protein Degradation	369
24.3	Polypharmacology of Thalidomide and its Derivatives	370
24.3.1	Anti-Myeloma Effects	371
24.3.2	Anti-MDS Effects	371
24.3.3	Anti-AML Effects	371
24.3.4	Immunomodulatory Effects	372
24.3.5	Teratogenic Effects	373
24.3.6	Proplatelet Formation Inhibition	374
24.4	Structural Understanding of the Mechanisms of Action of CELMoDs	374
24.4.1	The Interaction Between CRBN and Thalidomide	374
24.4.2	Formation of the Ternary Complexes: CELMoDs Acting as Molecular Glue	375
24.4.3	Allosteric Regulation for Neosubstrate Recognition	376
24.4.4	Expanding Neosubstrates of CRL4 ^{CRBN}	376
24.5	Challenges and Future Perspectives in the Development of CELMoDs	377
24.5.1	Advancing Drug Design: Molecular Glues and PROTACs	377
24.5.2	Physiological Functions of CRBN	377

24.5.3	Challenges of CELMoD Development From a Polypharmacological Perspective	378
24.5.4	Thalidomide and Beyond: Pursuing Non-CRBN Pathways for Hypnotic Action	379
24.6	Conclusions	379
	References	379
25	The Polypharmacology of Cariprazine and its Implications to Clinical Indications	385
	<i>Attila Egyed, Dóra J. Kiss, and György M. Keserű</i>	
25.1	Introduction	385
25.2	Structure and Binding	386
25.3	The Role of the Primary and Secondary Pharmacophore in Binding and Selectivity	387
25.4	Cariprazine–Functional Profile, Polypharmacology, and Functional Selectivity	389
25.5	<i>In Vivo</i> Profile of Cariprazine	390
25.6	Cariprazine in Clinical Practice	393
25.7	Conclusions	395
	References	396
26	Multi-Targeted Antivirals	405
	<i>Bing Ye, Letian Song, Meehyein Kim, Shenghua Gao, Peng Zhan, and Xinyong Liu</i>	
26.1	Multi-Target Inhibitors Targeting Both SARS-CoV-2 and Host Proteins	405
26.1.1	Multi-Target Inhibitors Targeting SARS-CoV-2 Protease and Host Targets	405
26.1.1.1	Targeting SARS-CoV-2 M ^{Pro} and Host Cathepsin L	405
26.1.1.2	Targeting SARS-CoV-2 M ^{Pro} and Spike Protein-ACE2 Interactions	408
26.1.1.3	Targeting SARS-CoV-2 M ^{Pro} and Host Protein Neuropilin-1 (NRP1)	409
26.1.1.4	Targeting SARS-CoV-2 M ^{Pro} and Human Phospholipase	409
26.1.1.5	Targeting SARS-CoV-2 M ^{Pro} and Human Inflammatory Factors	409
26.1.2	Multi-Target Inhibitors of Host Cell for Virus Entry	410
26.2	Multi-Target Inhibitors Directly Targeting SARS-CoV-2	411
26.2.1	Multiple-Targeting Inhibitors Towards SARS-CoV-2 RdRp	411
26.2.2	Multiple-Targeting Inhibitors Towards SARS-CoV-2 Proteases	414
26.2.3	Multi-Targeting SARS-CoV-2 Cell Entry Inhibitors Targeting Spike Proteins	416
26.3	Summary and Prospect	417
	Acknowledgments	418
	References	418
27	Multi-target Antimalarials as a Strategy to Reduce Resistance Risk	423
	<i>Lauren B. Coulson and Kelly Chibale</i>	
27.1	Introduction	423
27.2	Next-generation Antimalarials	424
27.3	Resistance Risk as a Criterion for the Prioritization of New Molecules and Targets	424
27.3.1	Assessing the Resistance Risk of Compounds in Development	424
27.3.2	Resistance Risk for Target-based Drug Discovery Programs	425
27.3.3	Irresistible Compounds	425
27.4	Polypharmacology in Malaria Drug Discovery	426
27.4.1	Dual Inhibition of <i>Plasmodium</i> Plasmepsin X and Plasmepsin IX	427
27.4.2	Dual-Site Inhibitors of the Cytochrome <i>bc_L</i> Complex	427
27.4.3	Multi-target Kinase Inhibitors for Malaria	429
27.4.3.1	<i>Plasmodium</i> Phosphatidylinositol 4-kinase Kinase Beta (PI4K β) Inhibitors	430
27.4.3.2	<i>Plasmodium</i> Cyclin-Dependent-Like Protein Kinase 3 (CLK3) Inhibitors	430
27.4.3.3	<i>Plasmodium</i> cGMP-dependent Protein Kinase (PKG) Inhibitors	431
27.4.3.4	Multi-target Kinase Inhibitors	431
27.5	Concluding Remarks and the Way Forward	432
	References	432

28	Multi-target Compounds for Tuberculosis	437
	<i>Giovanni Stelitano, Mario Cocorullo, and Laurent R. Chiarelli</i>	
28.1	Tuberculosis and the Problem of Antimicrobial Resistance	437
28.2	Polypharmacology to Fight <i>M. tuberculosis</i> Antimicrobial Resistance	438
28.3	Multitarget Compounds Against TB	439
28.4	Multitarget Compounds Against TB-HIV Co-infection	443
28.5	Conclusions	445
	References	445
29	Dual-acting HIV Inhibitors	451
	<i>María-José Camarasa, Ana-Rosa San-Félix, and Sonia de Castro</i>	
29.1	Introduction	451
29.2	HIV and Hepatitis Viruses Co-infections	451
29.2.1	Compounds with Dual Activity Against HIV and HBV Viruses	452
29.2.2	Compounds with Dual Activity Against HIV and HCV Viruses	454
29.3	Compounds with Dual Activity Against HIV and EV-A71	456
	Acknowledgement	458
	References	458
30	Multi-kinase Inhibitors for the Treatment of Pancreatic Cancer	463
	<i>Paul Dent and Andrew Poklepovic</i>	
	Acknowledgements	467
	References	467
	Index	469

List of Contributors

Tero Aittokallio

University of Helsinki
Finland/University of Oslo
Norway
tero.aittokallio@helsinki.fi

Alberto Ambesi-Impimbato

PsychoGenics
USA
alberto.ambesi@psychogenics.com

Jürgen Bajorath

Department of Life Science Informatics
B-IT LIMES Unit Chemical Biology and Medicinal
Chemistry
University of Bonn
Bonn
Germany
bajorath@bit.uni-bonn.de

Ian M. Bell

Merck & Co.
Discovery Chemistry
USA
ian_bell@merck.com

Eric A.G. Blomme

AbbVie Inc.
USA
eric.blomme@abbvie.com

Maria Laura Bolognesi

University of Bologna
Department of Pharmacy and Biotechnology
Italy
marialaura.bolognesi@unibo.it

Martin Bossart

Sanofi Germany
Integrated Drug Discovery Frankfurt
Germany
martin.bossart@sanofi.com

Daniela Brunner

PsychoGenics
Icahn School of Medicine at Mount Sinai
USA
Daniela.Brunner@psychogenics.com

Helmut Buschmann

Pharma Consulting Aachen
Germany
hbuschmann@gmail.com

María-José Camarasa

Instituto de Química Médica
Spain
mj.camarasa@iqm.csic.es

Laurent R. Chiarelli

University of Pavia
Department of Biology and Biotechnology
“Lazzaro Spallanzani”
Italy
laurent.chiarelli@unipv.it

Kelly Chibale

University of Cape Town
South Africa
kelly.chibale@uct.ac.za

Thomas Christoph

Translational R&D, Aachen
Germany
info@t-christoph.de

Anna Cichonńska

Harmonic Discovery
Finland
anna@harmonicdiscovery.com

Mario Cocorullo

University of Pavia
Department of Biology and Biotechnology
“Lazzaro Spallanzani”
Italy
mario.cocorullo01@universitadipavia.it

Lauren B. Coulson

University of Cape Town
South Africa
lauren.coulson@uct.ac.za

Sonia de Castro

Instituto de Química Médica
Spain
sonia@iqm.csic.es

Maedeh Darsaraee

University of Berne
Department of Chemistry
Biochemistry and Pharmacy
Switzerland
maedeh.darsaraee@unibe.ch

Paul Dent

Virginia Commonwealth University
Department of Biochemistry and Molecular Biology
USA
Paul.Dent@vcuhealth.org

Eleonora Diamanti

University of Bologna
Department of Pharmacy and Biotechnology
Italy
eleonora.diamanti2@unibo.it

Dang Ding

Shandong University
P.R. China
2429895062@qq.com

Attila Egyed

Research Centre for Natural Sciences
Medicinal Chemistry
Drug Innovation Centre
Hungary
egyed.attila@ttk.hu

Shenghua Gao

Shandong University
School of Pharmaceutical Sciences
Department of Medicinal Chemistry
Key Laboratory of Chemical Biology
P.R. China
shh_gao@163.com

Samaneh Goorani

University of Arkansas for Medical Sciences
USA
SGoorani@uams.edu

Jonathon R. Green

AbbVie Inc.
USA
jonathon.green@abbvie.com

Hiroshi Handa

Tokyo Medical University
Department of Molecular Pharmacology
Japan
hhanda@tokyo-med.ac.jp

Norbert Handler

RD&C Research
Development & Consulting GmbH
Austria
n.handler@rdc-concepts.com

Axel Helmstaedter

University of Marburg
Germany
helmstae@staff.uni-marburg.de

Gerhard Hessler

Sanofi Germany
Integrated Drug Discovery Frankfurt
Germany
gerhard.hessler@sanofi.com

John D. Imig

University of Arkansas for Medical Sciences
USA
jimig@uams.edu

Sacha Javor

University of Berne
Department of Chemistry
Biochemistry and Pharmacy
Switzerland
sacha.javor@unibe.ch

György M. Keserű

Research Centre for Natural Sciences
Medicinal Chemistry
Drug Innovation Centre
Hungary
keseru.gyorgy@ttk.hu

Meehyein Kim

Infectious Diseases Therapeutic Research Center
Korea Research Institute of Chemical Technology (KRICT)
Daejeon
Republic of Korea
mkim@krict.re.kr; sirnaworld@yahoo.com

Stephan Kirchner

F. Hoffmann-La Roche Ltd.
 Roche Pharma Research and Early Development
 Roche Innovation Center Basel
 Switzerland
 stephan.kirchner@roche.com

Dóra J. Kiss

Research Centre for Natural Sciences
 Medicinal Chemistry
 Drug Innovation Centre
 Hungary
 kiss.dora.judit@ttk.hu

Armando A. Lagrutta

Merck & Co.
 Nonclinical Drug Safety
 USA
 armando_lagrutta@merck.com

Xiangqian Li

State Key Laboratory of Microbial Technology
 Shandong University
 P.R. China
 lixiangqian@sdu.edu.cn

Helen L. Lightfoot

F. Hoffmann-La Roche Ltd.
 Roche Pharma Research and Early Development
 Roche Innovation Center Basel
 Switzerland
 helen_louise.lightfoot@roche.com

Felix F. Lillich

University of Frankfurt
 Germany
 lillich@pharmchem.uni-frankfurt.de

Xinyong Liu

Shandong University
 School of Pharmaceutical Sciences
 Department of Medicinal Chemistry
 Key Laboratory of Chemical Biology
 P.R. China
 xinyongl@sdu.edu.cn

Prathap Kumar S. Mahalingaiah

AbbVie Inc.
 USA
 prathapkumar.mahalingaiah@abbvie.com

Lee McDermott

PsychoGenics
 USA
 lee.mcdermott@psychogenics.com

Zeinab Mamdouh

Zagazig University
 Department of Pharmacology and Toxicology
 Egypt
 zmamdouh@ppmlab.net

Cristian Nogales

Maastricht University
 Department of Pharmacology and Personalised Medicine
 The Netherlands

and

Max Perutz Labs
 Vienna Biocenter Campus (VBC)
 Austria

and

University of Vienna
 Center for Molecular Biology
 Department of Structural and Computational Biology
 Austria
 cristian@menchelab.com

Arsenio Nueda

Almirall, S.A.
 Spain
 arsenio.nueda@almirall.com

Mayra Pacheco Pachado

Maastricht University
 Pharmacology and Personalised Medicine
 The Netherlands
 mpachado@ppmlab.net
 mayra.pachado@gmail.com

Anna M. Pasieka

University of Bologna
 Department of Pharmacy and Biotechnology
 Italy
 pasieka.ann@gmail.com

Alan Lars Pehrson

PsychoGenics
 USA
 alan.pehrson@psychogenics.com

Jens-Uwe Peters

Skyhawk Therapeutics
 Switzerland
 Jup1@live.com

Zina Piper

Maastricht University
 Pharmacology and Personalised Medicine
 The Netherlands
 zpiper@ppmlab.net

Andrew Poklepovic

Virginia Commonwealth University
Department of Medicine
USA
andrew.poklepovic@vcuhealth.org

Michal Poznik

RD&C Research
Development & Consulting GmbH
Austria
m.poznik@rdc-concepts.com

Ewgenij Proschak

University of Frankfurt
Germany
proschak@pharmchem.uni-frankfurt.de

Rayees Rahman

Harmonic Discovery
USA
rayees@harmonicdiscovery.com

Balaguru Ravikumar

Harmonic Discovery
Finland
guru@harmonicdiscovery.com

Jean-Louis Reymond

University of Berne
Department of Chemistry
Biochemistry and Pharmacy
Switzerland
jean-louis.reymond@unibe.ch

Sonia Roberts

F. Hoffmann-La Roche Ltd.
Roche Pharma Research and Early Development
Roche Innovation Center Basel
Switzerland
sonia.roberts@roche.com

Navriti Sahni

Harmonic Discovery
USA
navriti@harmonicdiscovery.com

Ana-Rosa San-Félix

Instituto de Química Médica
Spain
anarosa@iqm.csic.es

Tiffany Schwasinger-Schmidt

University of Kansas School of Medicine-Wichita
Department of Internal Medicine
USA
tschwasinger-schmidt@kumc.edu

Dayong Shi

State Key Laboratory of Microbial Technology
Shandong University
P.R. China
shidayong@sdu.edu.cn

Lynn L. Silver

LL Silver Consulting
USA
llsilverconsulting@gmail.com

Letian Song

Shandong University
School of Pharmaceutical Sciences
Department of Medicinal Chemistry
Key Laboratory of Chemical Biology
P.R. China
kevinsong319@163.com

Giovanni Stelitano

University of Pavia
Department of Biology and Biotechnology
“Lazzaro Spallanzani”
Italy
giovanni.stelitano@unipv.it

Kai Tang

Shandong University
P.R. China
13027750680@163.com

Peter L. Toogood

University of Michigan
USA
toogood@umich.edu

Elisa Uliassi

University of Bologna
Department of Pharmacy and Biotechnology
Italy
elisa.uliassi3@unibo.it

Terry R. Van Vleet

AbbVie Inc.
USA
terry.vanvleet@abbvie.com

Andy Vo

AbbVie Inc.
USA
Andy.vo@abbvie.com

Shujing Xu

Shandong University
P.R. China
xu17854111942@163.com

Yuki Yamaguchi

Tokyo Institute of Technology
School of Life Science and Technology
Japan
yyamaguc@bio.titech.ac.jp

Junichi Yamamoto

Tokyo Institute of Technology
School of Life Science and Technology
Japan
yamamoto.j.ab@m.titech.ac.jp

Bing Ye

Shandong University
School of Pharmaceutical Sciences
Department of Medicinal Chemistry
Key Laboratory of Chemical Biology
P.R. China
2860322431@qq.com

Dimitar Yonchev

F. Hoffmann-La Roche Ltd.
Roche Pharma Research and Early Development
Data & Analytics
Roche Innovation Center Basel
Switzerland
dimitar.yonchev@roche.com

Peng Zhan

Shandong University
School of Pharmaceutical Sciences
Department of Medicinal Chemistry
Key Laboratory of Chemical Biology
P.R. China
zhanpeng1982@sdu.edu.cn

Yang Zhou

Shandong University
P.R. China
13503806229@163.com

Preface

Many drugs act on more than one target [1]. This can be necessary for efficacy, but can also lead to adverse effects [2]. For instance, it was discovered in the 1980s that dual $D_{2/3}$ and $5-HT_{2a}$ receptor antagonism is needed for efficacy in antipsychotic drugs [3]. Today we know that antipsychotics bind to more than 20 targets, some of which contribute to efficacy, but also cause adverse effects [4].

In the early 2000s, the term polypharmacology was introduced to describe this concept of drugs binding to several targets. During this time, it became increasingly recognized that multi-target activity is often needed for efficacy. For instance, the antibiotic research of the 1990s focused on single targets derived from bacterial genomes. These single-targeted approaches were generally fruitless and did not lead to new drugs. Instead, nearly all systemically efficacious antibiotics bind to multiple targets or to targets encoded by multiple genes, so that single mutations do not lead to drug resistance (further discussed in Chapter 10) [5]. It was also recognized that unintended “anti-target” activity leads to adverse effects. Here, the most prominent example is an unusual high number of drugs withdrawn from the market in 1996–2001. These drugs were withdrawn due to adverse effects, which could be traced back to anti-target activity. For instance, half a dozen of drugs was withdrawn due to their potential to cause cardiac arrhythmias caused by unintended blockade of the hERG channel (see Chapter 7) [6]. Thus, polypharmacology can be beneficial or detrimental – these two sides of the polypharmacology coin are further detailed in the introduction.

Following some widely read papers on concepts such as “Network Pharmacology” [7] or “Magic Shotguns” [8], polypharmacology became an increasingly popular research topic. From 2010 onward, Scifinder searches retrieve an ever-increasing number of publications on polypharmacology and related topics, such as multi-target, off-target, and secondary or network pharmacology. A first book on polypharmacology was published in 2012 and became a popular read [9]. This current book is a follow-up with an updated and expanded content.

The book is divided into four parts A–D. Part A discusses undesired polypharmacology, which is often a safety concern. For instance, many drugs bind to “anti-targets” or “off-targets”, e.g. to cardiac ion channels. This causes adverse effects such as cardiac arrhythmia. The relevance of such anti-targets for adverse effects will be discussed in a first chapter, followed by chapters on the link between off-targets and adverse drug reactions, on how to screen for off-target activity and how to recognize and optimize compounds with a potential for off-target activity. This is followed by a discussion of kinases and cardiac ion channels, two of the most important classes of anti-targets.

The remainder of the book is dedicated to intended polypharmacology. Part B discusses disease areas, which benefit from polypharmacological approaches. A first chapter outlines the general concept of network pharmacology and multi-target drugs. The following chapters focus on oncology, bacterial and viral infections, CNS diseases, and metabolic diseases, followed by a discussion of the role of polypharmacology in the history of drug discovery.

But how can we discover such multi-target drugs? Part C of the book highlights important approaches, such as compound design, data mining with web-based tools, multi-target peptides, as well as phenotypic screening in cells, tissues, and animal models. A related topic is the Selective Optimization of Side Effects (SOSA) approach to drug discovery, which will be discussed as well.

The final Part D collects case studies on polypharmacological drugs and current research. PROTACs and molecular glues are hot topics in drug discovery, and the first chapter outlines how these originate from the polypharmacology of thalidomide. Next is a story on achieving “selective dual activity” for cyclin-dependent kinase inhibitors. This is followed by a bouquet of topics, from the discovery of cariprazine and tapentadol, to current research on antivirals, malaria, tuberculosis, HIV, and pancreatic cancer.

This book on polypharmacology is intended as a comprehensive resource for industrial drug hunters and academic

researchers. It illuminates all facets of polypharmacology, from anti-target screening, to the design of multi-target ligands. A comparison of the current book with the first book from 2012 [9] shows that polypharmacology has certainly come of age. Polypharmacology research has improved the drug discovery process, has delivered ideas for Biotech Startups, and has garnered the attention of the media [10]. Hopefully, this book will inspire readers for new drug discovery projects and will help to mitigate attrition due to safety issues.

I am very grateful to all contributing authors, who invested their time and their expertise into this book. Also, I thank the team at Wiley for proposing this book and for their advice throughout this project: Katherine Wong, Jonathan Rose, Sabeen Aziz, Shwathi Srinivasan, and Keerthana Baskaran.

Enjoy reading!

Jens-Uwe Peters

Basel, Switzerland, December 2024

References

- 1 Hu, Y. and Bajorath, J. (2013). Compound promiscuity: what can we learn from current data? *Drug Discov. Today* 18 (13/14): 644–650.
- 2 Peters, J.-U. (2013). Polypharmacology – Foe or Friend? *J. Med. Chem.* 56 (22): 8955–8971.
- 3 Meltzer, H.Y., Matsubara, S., and Lee, J.C. (1989). Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin₂ pKi values. *J. Pharmacol. Exp. Ther.* 251 (1): 238–246.
- 4 Riemer, C. (2012). Antipsychotics. In: *Polypharmacology in Drug Discovery* (ed. J.-U. Peters), 343–362. Hoboken: Wiley.
- 5 Silver, L.L. (2012). Polypharmacology as an Emerging Trend in Antibacterial Discovery. In: *Polypharmacology in Drug Discovery* (ed. J.-U. Peters), 167–202. Hoboken: Wiley.
- 6 Bell, I.M., Bilodeau, M.T., and Lagrutta, A.A. (2012, 2012). Activity at Cardiovascular Ion Channels: A Key Issue for Drug Discovery. In: *Polypharmacology in Drug Discovery* (ed. J.-U. Peters), 83–109. Hoboken: Wiley.
- 7 Hopkins, A.L. (2008). Network pharmacology: the next paradigm in drug discovery. *Nat. Chem. Biol.* 4 (11): 682–690.
- 8 Roth, B.L., Sheffler, D.J., and Kroeze, W.K. (2004). Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discovery* 3 (4): 353–359.
- 9 Peters, J.-U. (ed.) (2012). *Polypharmacology in Drug Discovery*. Wiley: Hoboken.
- 10 Kurji, N. (2019). The master key to drug design: multi-target drugs. <https://www.forbes.com/sites/forbestechcouncil/2019/09/16/the-master-key-to-drug-design-multi-target-drugs/?sh=54ba77576cfe> (accessed 27 September 2024).

1

Introduction

Facets of Polypharmacology – a Janus-Headed Concept for Drug Discovery

Jürgen Bajorath

1.1 Origins

Since the 1980s, target-centric approaches have dominated drug discovery efforts, triggered by the molecular-biology-driven reductionist approach [1] and leading to the “one drug, one target,” or “drug specificity” paradigm [2]. Molecular reductionism aimed at “dissecting biological systems into their constituent parts” [1]. Different from the preceding more holistic and pharmacology-oriented era in drug discovery, molecular sciences and the single-target (ST) focus took the centerstage and shaped drug discovery efforts for many years to come [1, 2]. These developments were paralleled by advances in X-ray crystallography and molecular graphics catalyzing a wave of structure-based (“rational”) drug design efforts [3, 4], which further emphasized the focus on target-specific compounds in drug discovery.

In the early 2000s, systems biology emerged [5] and also entered the drug discovery arena [6] introducing, for example, network representations of biological systems, pathway modeling, and computational disease models. These developments originating from bioinformatics also altered the view of traditional disciplines such as pharmacology, giving rise to a network perception of physiological processes and increasing the notion of their interdependence [7]. In pharmacological networks, multiple signaling and metabolic pathways establish functional links and dependencies between different target proteins [7, 8]. If pathways in such networks are perturbed or regulatory and control mechanisms compromised, different types of multifactorial diseases might arise, including various forms of cancer, complex diseases of the central nervous system, or metabolic diseases [9–12]. Such diseases could most likely not be effectively treated by therapeutic intervention of individual targets, but required multi-target (MT) engagement instead, thus departing from the target specificity paradigm in drug discovery. MT activity of drugs was not

unknown and probably first observed for anti-psychotics and antiepileptics beginning in the late 1980s [12, 13].

In 2006, as a consequence of the increasing notion of pharmacological networks, the concept of polypharmacology was introduced [14], focusing on MT agents for the treatment of multifactorial diseases: “*Contrary to the dogma that the ‘rational’ way to discover drugs is to design exquisitely selective ligands for single molecular targets, a rival hypothesis proposes polypharmacology or the promiscuous modulation of several molecular targets*” [14]. In 2014, a formal definition of polypharmacology appeared in the US National Library of Medicine (NLM) as “*the design or use of pharmaceutical agents that act on multiple targets or disease pathways*.” Polypharmacology also encompasses the pharmacological effects resulting from the use of MT compounds (MT-CPDs), consistent with the principles of network pharmacology. MT activity of drugs and other bioactive compounds is often also referred to as “promiscuity” (not to be confused with nonspecific compound–protein interactions).

1.2 Pros and Cons

Following its inception, polypharmacology emerged as an alternative to reductionist approaches and rational drug design and further evolved into a multifaceted drug discovery strategy [15–17], albeit “Janus-headedly.” In Roman mythology, Janus, the god of the beginnings, passages, and endings, had two opposing faces. Accordingly, the “Janus head” became a symbol of duality and ambivalence – which exactly applied to the polypharmacology concept: on the one hand, MT activity of drugs is a prerequisite for therapeutic efficacy in the treatment of multifactorial diseases; on the other, it is responsible for unwanted (adverse) side effects [15, 18, 19]. While adverse side effects can be elicited by the engagement of a primary target, they are more frequently caused by inhibiting so-called anti-targets

such as cardiac ion channels (hERG), drug-metabolizing cytochrome P450 isoforms, or G-protein-coupled neurotransmitter receptors [15, 16]. Furthermore, side effects of MT-CPDs might also be caused by interacting with other targets not implicated in immediate toxicity, due to pathway modulations. In the pharmaceutical industry, potential liabilities as a consequence of interactions with anti-targets are a major concern, for example, leading to the assessment of newly identified candidate compounds in various safety screens for activity against such targets. However, not all unexpected side effects are undesired, taking into consideration that MT activity also provides the basis for drug repurposing [20]. Benefits of MT activity of drugs were often discovered post hoc. For example, adenosine triphosphate (ATP)-site-directed kinase inhibitors used in cancer therapy were originally thought to be kinase-selective, based on reductionist assessment, before it was discovered that their clinical efficacy depended on multi-kinase activity and simultaneous interference with multiple deregulated signaling pathways [21]. This also applied to imatinib, the first kinase inhibitor marketed as a drug [21].

Despite the Janus-headed nature of polypharmacology and the risks associated with potential adverse side effects resulting from the MT activity of drugs, the positive impact of polypharmacology on drug discovery and development is undeniable, as demonstrated by the continuous occurrence of MT agents among newly approved drugs. For example, 10 of 49 European Medicines Agency (EMA)-approved drugs marketed in Germany in 2022 were annotated with two or more targets [22]. Of course, despite the strong impact of polypharmacology, the development of compounds with target selectivity or specificity continues to be a pillar of drug discovery and development. For example, for long-term treatment of chronic and non-life-threatening diseases, drug side effects must inevitably be minimized, rendering target-selective compounds highly desirable.

1.3 Discovery and Design

Similar to coincidental findings that side effects of drugs originally thought to be specific were caused by previously unknown secondary targets, new MT-CPDs are often discovered serendipitously, for example, in screening campaigns or target deconvolution of active compounds from phenotypic assays. Given the high interest in compounds with defined MT activity in different therapeutic areas, prospective design of such compounds is also a topical issue in drug discovery [23, 24]. However, consistent with findings that characteristic structural features of MT-CPDs generally depend on target combinations, as further discussed below, the prospective design of MT-CPDs

with desired activity is challenging, mostly carried out on a case-by-case basis in medicinal chemistry and far from being routine. For all practical purposes, prospective design of MT-CPDs for polypharmacology is limited to two or at most three targets. To this end, combining or merging target-dependent pharmacophores is a popular knowledge-based approach for MT-CPD design that is readily applicable in the practice of medicinal chemistry and does not require sophisticated computations [23–25]. Pharmacophore fusion attempts can be further extended by screening of test compounds using pharmacophore models for different targets and follow-up analysis of shared hits [26]. As an alternative to pharmacophore modeling, scaffolds isolated from compounds with known activity against different targets can also be used as templates for MT-CPD design, as further discussed below.

In addition to knowledge-based design strategies that are close to practical medicinal chemistry, other ligand- or target-structure-based computational approaches have been applied to identify compounds for polypharmacology [27, 28]. For example, various machine learning (ML) models have been reported to distinguish between compounds with MT activity and corresponding ST activity (typically achieving reasonable to high prediction accuracy). Furthermore, ML models have been used for computational target profiling. Here, test compounds are virtually screened using large numbers of individually derived target-based models to predict MT-CPDs. As a deep learning alternative, multitask models have also been developed to predict compounds with activity against related targets. At the structural level, similarities of binding sites in different targets have been quantified as an indicator of polypharmacology potential at the target level. In addition, parallel docking campaigns or cross-docking screens have been carried out for structure-based target profiling. Furthermore, ligands bound to different proteins have been systematically compared to identify compound pairs with the highest shape similarity to prioritize and evaluate putative cross-target activities [28].

1.4 Structural Data

In addition to its relevance for polypharmacology, the study of MT-CPDs is also of interest from a basic scientific perspective. For example, exploring the mechanisms by which small molecules “multi-specifically” or “pseudo-specifically” interact with different targets helps to better understand these special molecular recognition phenomena. To this end, currently available X-ray structures of complexes formed by MT-CPDs and different proteins provide substantial information. For example, in 2018, we identified 1418 crystallographic MT-CPDs

(>300 Da) in X-ray structures of complexes with different targets available in the Protein Data Bank (PDB) [29, 30]. These MT-CPDs included 702 ligands forming complexes with targets from different protein families (termed multifamily ligands) [30]. Bound conformations of multifamily ligands available in complexes with unrelated targets were compared in detail, revealing a variety of ligand binding modes [31]. In some instances, these ligands conformationally adapted to binding sites having different architectures and chemical features and displayed different binding modes; in others, binding modes were surprisingly conserved in differently shaped active sites. If binding modes of multifamily ligands were conserved, characteristic interaction patterns emerged for targets from a given family that differed from others, hence providing a possible rationale for the conservation of binding modes [31].

As a representative example, Figure 1.1 shows conserved and variable binding modes in different active sites for indomethacin, a nonsteroidal anti-inflammatory drug (NSAID) with known polypharmacology used for the treatment of acute pain and symptoms of osteoarthritis and rheumatoid arthritis.

For 243 of the 702 multifamily ligands, 168 analogue series were detected in the ChEMBL database [32]. These series consisted of a total of 4829 compounds, covered 190 additional targets, and yielded 133 unique analogue series-based scaffolds [30]. Figure 1.2 shows an exemplary

scaffold. All analogue series scaffolds were annotated with different target combinations, providing a knowledge base of MT template compounds.

1.5 Activity Data

Rapidly growing volumes of compound activity data provide another information-rich resource for the study of MT-CPDs and polypharmacology. Since the analysis of MT activity is particularly vulnerable to false-positive activity annotations, compound activity data should be carefully curated and potential assay interference compounds [33, 34] or colloidal aggregators [35] should be removed. Indeed, results of MT activity analysis strongly depend on applied data confidence criteria [36], as illustrated in Figure 1.3 for imatinib, suggesting to restrict the assessment of MT-CPDs to high-confidence activity data [36].

There are different facets of MT activity. For instance, it is not very surprising that some active compounds exhibit a tendency to interact with more than one closely related target, such as ATP-site-directed protein kinase inhibitors. By contrast, compounds binding to structurally and functionally unrelated proteins are rather unexpected, but of special interest, from both a basic scientific and a polypharmacology perspective. For example, such compounds might interfere with distinct physiological functions and elicit

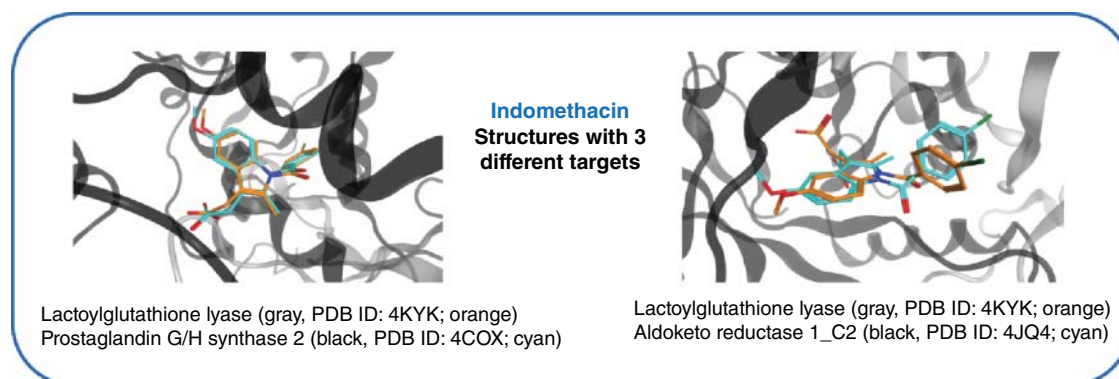
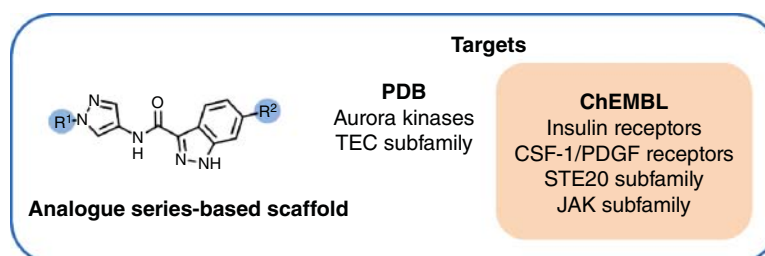


Figure 1.1 X-ray structures of indomethacin in complex with three distinct targets. On the left, and right, pairwise superpositions of bound ligand conformations are shown, revealing conserved (left) and variable binding modes (right) in different protein environments.

Figure 1.2 Scaffold of a multifamily ligand with kinase activity representing an analogue series. For the ligand, crystal structures of complexes with Aurora and TEC kinases were available (PDB) and structural analogues found in ChEMBL were active against additional kinase targets from other families.



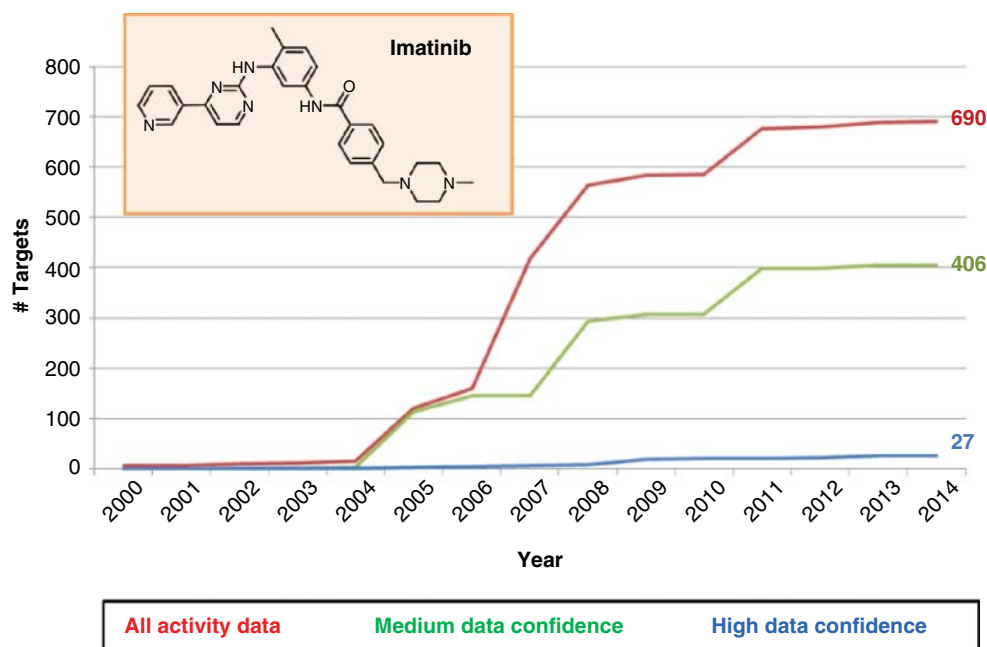


Figure 1.3 Target annotations of imatinib. Based on increasing volumes of activity data from ChEMBL collected over time, the number of targets reported for imatinib is monitored at three different confidence levels: all activity data (no confidence criteria were applied) medium- and high-confidence data. Adapted from Hu and Bajorath [36]. The number of target annotations based on all activity data and medium-confidence data (690 and 406, respectively) is unrealistic.

unexpected pharmacological effects. Systematic analysis of compound activity data helps to estimate the frequency with which MT-CPDs occur and the number of targets they are active against. Especially for candidate compounds and drugs, such estimates are relevant to balance often articulated expectation values that are largely unsubstantiated (e.g., “most drugs bind to 10 or 20 targets ...”). In addition, careful analysis of available compounds and activity data also helps to gauge predictions of MT-CPDs and their target numbers, for example, from computational target profiling (*vide supra*). Notably, compound-data-driven analysis principally underestimates MT activity due to data incompleteness, given that not “all compounds have been tested against all targets” (the ultimate goal of chemogenomics). This must be taken into consideration. On the other hand, analysis of the large and rapidly growing volumes of activity data available in the public domain should reveal some statistically sound trends [36]. For instance, in 2019, we carried out a large-scale analysis of biological screening data from PubChem [37] in the search for compounds with activity against targets from different classes [38]. A total of 1063 compounds were identified that were tested in assays for at least 100 human target proteins and were active against at least 10 targets from more than one class [38]. These findings showed that MT-CPDs with activity against distantly or unrelated targets occurred rather frequently.

1.6 Drug Target Estimates

Systematic experimental determination of the targets that drugs are active against is far from being an easy task. Accordingly, insights into drug target numbers are typically confined to case-by-case proteomic analysis or statistics from target panel assays such as kinome screens [39]. However, based on compound data analysis, different estimates of target numbers for drugs and other active compounds have been reported.

Early attempts to predict drug targets used network representations of drug–target interactions [40]. From different databases, drugs, targets, and interaction data were collected and analyzed in drug–target networks. From such network representations, it was estimated that a drug on average interacted with six targets. Depending on the data used, targets per drug ranged from approximately 3 to 13 [40]. Comparable estimates were obtained when approved and experimental drugs taken from DrugBank [41] were mapped to ChEMBL and drug data and targets were monitored over a 15-year period [42]. For bioactive compounds from screening assays, different target numbers were determined. In an early analysis of PubChem [37], MT activities were analyzed on the basis 600+ assays [43]. It was found that approximately 58% of active screening compounds only displayed ST activity in combined primary and confirmatory assays. In addition, based on high-confidence