# 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

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



***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

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

***Alberto Ambesi-Impiombato***
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 Cichońska***
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

*Polypharmacology: Strategies for Multi-Target Drug Discovery,* First Edition. Edited by Jens-Uwe Peters.

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

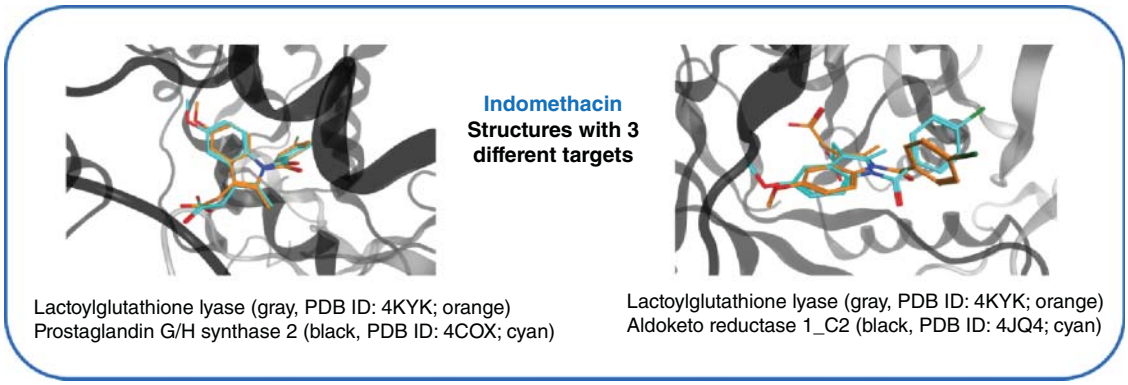
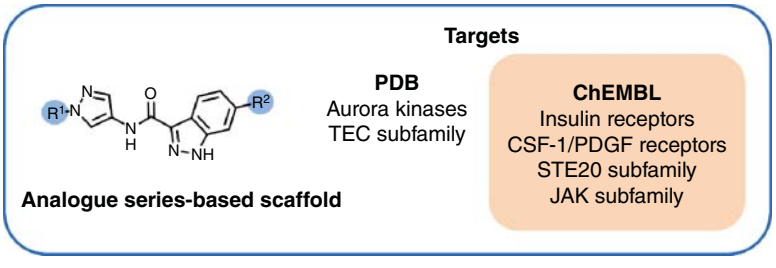



**Figure 1.1** X-ray structures of indomethacin in complex with three distinct targets. On the left, and right, pairwise superpositions of bound ligand conformations are shown, revealing conserved (left) and variable binding modes (right) in different protein environments.

**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.



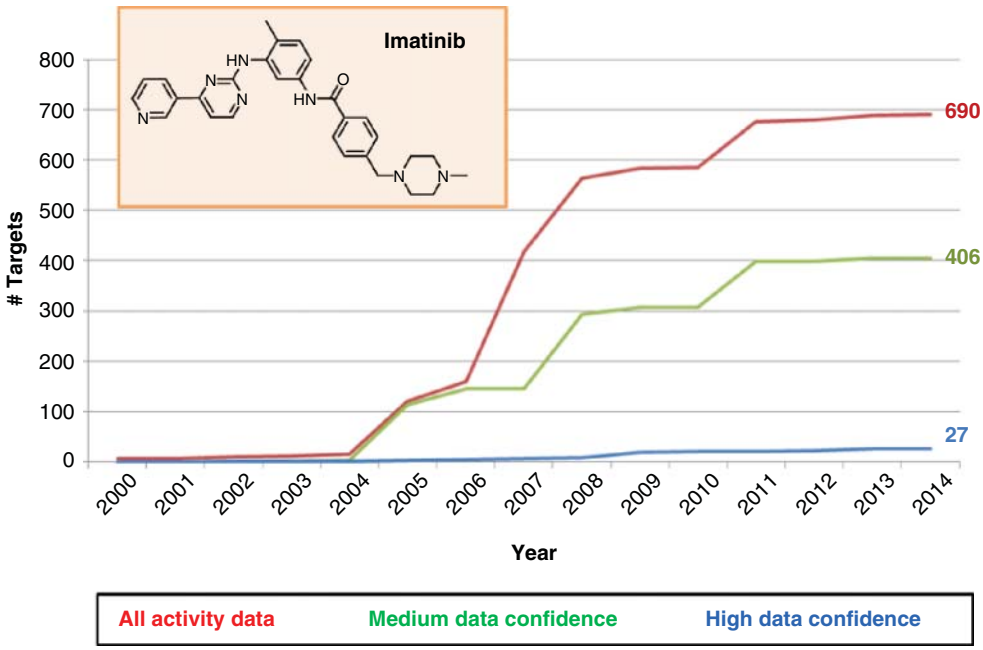



**Figure 1.3** Target annotations of imatinib. Based on increasing volumes of activity data from ChEMBL collected over time, the number of targets reported for imatinib is monitored at three different confidence levels: all activity data (no confidence criteria were applied) medium- and high-confidence 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