# Drug Metabolism and Pharmacokinetics

## Frontiers, Strategies, and Applications

Edited by Liang Shen and WuXi AppTec DMPK

WILEY

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



The manufacturer's authorized representative according to the EU General Product Safety Regulation is Wiley-VCH GmbH, Boschstr. 12, 69469 Weinheim, Germany, e-mail: Product_Safety@wiley.com



***Library of Congress Cataloging-in-Publication Data applied for:***
Hardback ISBN: 9781394300129
ePDF ISBN: 9781394300143
epub ISBN:9781394300136

Cover Design: Wiley
Cover Image: Created by and Courtesy of WuXi AppTec

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

# Contents

## About the Editors

***Liang Shen***

Ph.D., Vice President, Head of DMPK Department, WuXi AppTec

Dr. Liang Shen has more than 20 years of experience in drug metabolism and pharmacokinetics (DMPK), toxicology, clinical pharmacology, and precision medicine. Dr. Shen has published or co-authored over 50 research articles and has led or participated in the compilation or translation of four books in drug discovery or DMPK field. His extensive experience in drug discovery, preclinical candidate (PCC) profiling, and investigational new drug (IND) submissions has successfully supported multiple pipelines in the fields of oncology, immuno-oncology, immunology, metabolic diseases, infectious diseases, central nervous system (CNS), and cardiovascular diseases to proof-of-concept at the early clinical stage. He received his PhD degree from the University of Georgia in the United States.

***WuXi AppTec DMPK***

As a global company with operations across Asia, Europe, and North America, WuXi AppTec provides a broad portfolio of R&D and manufacturing services that enable the global pharmaceutical and life sciences industry to advance discoveries and deliver groundbreaking treatments to patients. The Department of DMPK of WuXi AppTec was established in 2006 and offers *in vivo* and *in vitro* DMPK services covering stages from discovery screening to preclinical development and clinical studies. With research facilities located in the United States (New Jersey) and China (Shanghai, Suzhou, Nanjing, and Nantong), the department has supported thousands of investigational new drug (IND) and new drug applications (NDA). The services of WuXi AppTec DMPK include *in vitro* absorption, distribution, metabolism, excretion (ADME) studies, *in vivo* pharmacokinetic studies, metabolite profiling and identification studies, synthesis of radiolabeled compounds, radiolabeled ADME including human absorption, metabolism, and excretion (AME) studies, and bioanalysis. For more information, visit dmpkservice.wuxiapptec.com.

# Preface

Drug metabolism and pharmacokinetics (DMPK) studies involve characterizing the absorption, distribution, metabolism, and excretion (ADME) properties of compounds. This aids in identifying drugs with enhanced safety and potency, minimizing the risk of drug-drug interactions, and establishing a foundation for determining clinical dosage and frequency. The advancement of the pharmaceutical industry and the emergence of novel drug modalities have presented new challenges and opportunities for drug development, driving continuous innovation and progress in DMPK research.

This book is written by a team of scientists specializing in DMPK research from the DMPK Department of WuXi AppTec. The team was established in 2006 and has successfully supported thousands of investigational new drug (IND) applications globally. Drawing on a wealth of extensive practical experience and theoretical research, this book encapsulates the most recent advancements and illustrative applications. It is intended for pharmacy students, pharmaceutical industry researchers, and DMPK scientists, especially those exploring novel drug modalities.

Sixty-eight relatively independent yet interconnected articles compose this book, each offering a unique perspective and providing in-depth interpretation. Readers can either read systematically or select specific topics of interest from the table of contents. Basic concepts, frontier advancements, DMPK research strategies, and technical methods are covered in the book for novel drug modalities, and therapeutics in different disease areas. Furthermore, the book encompasses a wide range of application and validation cases for DMPK research, including studies in *in vitro* ADME, *in vivo* pharmacokinetics, metabolite profiling and identification, radiolabeled ADME, and bioanalysis.

The book is divided into three sections. Section I explores the pharmacokinetic strategies and research advancements in novel drug modalities, including proteolysis-targeting chimeras (PROTACs), antibody-drug conjugates (ADCs), peptide-drug conjugates (PDCs), peptide drugs, oligonucleotide (OLIGO) drugs, and mRNA-based vaccines and therapeutics. The mechanisms of these new drug modalities impose higher requirements on their DMPK studies. For example, ADC drugs are composed of antibodies and small molecule toxins and exhibit both large molecule and small molecule characteristics, making their *in vivo* process complicated and dynamic with biotransformation and drug-to-antibody ratio (DAR) changes. This complexity poses challenges to ADC studies such as stability, metabolite identification, and bioanalysis.

Section II focuses on the drug metabolism and pharmacokinetic studies of therapeutics in specific disease areas. With an aging population and an increasing number of patients with chronic diseases, there is a growing demand for drug development in disease areas with unique pharmacokinetic characteristics, such as ophthalmic drugs, respiratory medications, transdermal and topical drugs, and central nervous system drugs. Specific preclinical study models or methods

need to be developed to address these characteristics resulting from different pathogenesis and therapeutic modes of action. For example, developing ophthalmic drugs will encounter difficulties such as multiple biological barriers, analytical complexities, and intricate experimental operations.

Section III delves into front-edge metabolism and pharmacokinetic research strategies, methods, and applications, covering topics such as *in vitro* ADME, *in vivo* pharmacokinetics, metabolite profiling and identification, radiolabeled ADME studies, and advanced bioanalysis techniques and platforms. These approaches enable more comprehensive and accurate drug evaluation in DMPK studies.

Scientists are devoted to drug metabolism and pharmacokinetic studies, and expand the breadth and depth of their research by summarizing and optimizing the methodologies and insights from massive experience. Nevertheless, it is essential to be aware that the DMPK research will be full of opportunities and challenges with technological advancements. Continuous investment of time and resources is essential to keep pace and add to clinical breakthroughs and health sciences.

The publication of this book aims to bring together the knowledge and experience from WuXi AppTec DMPK's forefront scientists, and contribute to new drug development in this era of industrial transformation. I sincerely thank the authors for their efforts and contributions, as well as Wiley for their review and support. I hope readers can enjoy reading while finding useful tactics in this book.

Dr. Liang Shen
Vice President, Head of DMPK Department
WuXi AppTec

## Acknowledgments

The compilation and publication of this book have been a joint effort. Credit should be given to the authors and editors, who have delivered profound insights by drawing upon their vast DMPK expertise. Their hard work, research, and commitment in crafting this book deserve special recognition. We would like to thank all the authors and editors, listed here in alphabetical order.

Peiyun An, Weiqun Cao, Dr. Genfu Chen, Dr. Jacob Zhi Chen, Dr. Shiyan Chen, Song Chen, Yu Chen, Liwei Cheng, Dr. Qigan Cheng, Hongfang Cui, Qian Cui, Tiantian Dang, Ya Ding, Xuan Dong, Jian Fang, Quanli Feng, Flora Fu, Xinfa Fu, Li Gao, Lijia Gao, Yafei Gao, Yang Gao, Lian Guo, Qiqi Han, Huan He, Lijuan Hou, Jie Hu, Weimin Hu, Dr. Jiaming Jiang, Dr. Lifang Jiang, Dr. Furong Jiao, Dr. Jing Jin, Wei Lei, Chengyuan Li, Hongye Li, Huan Li, Huayu Li, Dr. Huihui Li, Jie Li, Lin Li, Dr. Peng Li, Qian Li, Ruixing Li, Tingting Li, Xiaotong Li, Xin Li, Yingying Li, Zhihai Li, Dr. Zhiyu Li, Danqing Lin, Haijuan Liu, Dr. Huan Liu, Huan Liu, Jian Liu, Mengjie Liu, Qing Liu, Shoutao Liu, Siyu Liu, Yanfeng Liu, Chunhong Lu, Jinlian Lu, Yikuan Lu, Dr. Dongke Ma, Dr. Liping Ma, Chen Ning, Jie Pan, Dr. Yan Pan, Linlin Pei, Hongfeng Qian, Huijuan Qian, Gengyao Qin, Dr. Li Qu, Xiang Ren, Yanfu Ren, Tingting Ruan, Dr. Liang Shen, Dr. Liqi Shi, Xinmeng Shi, Miaomiao Song, Yuanqiang Su, Hong Sun, Jianping Sun, Ziqian Sun, Cheng Tang, Liping Tang, Xue Tang, Dr. Yi Tao, Binbin Tian, Dr. Hongmei Wang, Huijuan Wang, Dr. Jie Wang, Junjie Wang, Dr. Xiangling Wang, Dr. Xiaoqi Wang, Yu Wang, Yuxi Wang, Xinxin Wen, Lili Xing, Haiwei Xiong, Tao Xiong, Dr. Mingcheng Xu, Ye Xu, Jia Xue, Dr. Huan Yan, Jinyu Yan, Dr. Yunyi Yan, Guang Yang, Rong Yang, Xianqing Yu, Dr. Jiaqi Yuan, Chao Zhang, Hefeng Zhang, Dr. Hong Zhang, Honghong Zhang, Dr. Lingling Zhang, Xuan Zhang, Yingying Zhang, Yu Zhang, Hang Zhao, Dr. Nan Zhao, Lijin Zheng, Dr. Maotian Zhou, Xinyun Zhou, Ying Zhou, Wenjun Zhu, and Zhangpei Zhu.

# Section I

# Novel Drug Modalities: Advancements and Their Pharmacokinetic Strategies

# 1

# Proteolysis-Targeting Chimeras (PROTACs)

## 1.1 An Overview of PROTAC Technology and Drug Metabolism and Pharmacokinetic (DMPK) Research Strategies

*Chengyuan Li, Yu Wang, Jing Jin*

### 1.1.1 Introduction

In recent decades, significant advances have been achieved in the development of new therapeutic approaches. Proteolysis-targeting chimera (PROTAC) offers a new approach to some disease treatments, which has increasingly attracted the attention of drug developers in the last five years. Additionally, PROTAC, as a new therapeutic technology, has facilitated substantial investment worldwide. In this chapter, we summarized the PROTAC's structure and mechanism of action, its global landscape, the drug metabolism and PK (DMPK) challenges in PROTAC research, and corresponding potential solutions.

### 1.1.2 Structure and Mechanism of Action of PROTACs

PROTAC utilizes the body's natural protein degradation mechanism, known as the ubiquitin–proteasome system [1]. PROTAC is a bifunctional molecule that consists of three key structural parts: a ligand that binds the target protein, a ligand that binds the ubiquitin–protein ligase (E3), and a linker that connects the two ligands (Figure 1.1).

Because of its unique structure, PROTAC brings the E3 ligase and the target protein to proximity, which induces the E3 ligase to label the target protein with ubiquitin. This causes the target protein to degrade [2]. The PROTAC molecules that detach after the degradation of the target protein can be recycled (Figure 1.2). In contrast to the occupancy-driven pharmacology of most small-molecule drugs, PROTAC can access proteins that were previously inaccessible without occupying an active pocket or relying on target occupancy to disrupt the function of the target protein. This is known as event-driven pharmacology.

Therefore, PROTAC offers many advantages in drug discovery:

- It has the potential to target many undruggable protein targets that lack active sites.
- While retaining the advantage of directly targeting intracellular proteins, it has the potential to overcome some of the disadvantages of small molecules such as drug resistance.
- To improve patient compliance, it can be administered orally.

*Drug Metabolism and Pharmacokinetics: Frontiers, Strategies, and Applications*, First Edition.
Edited by Liang Shen and WuXi AppTec DMPK.

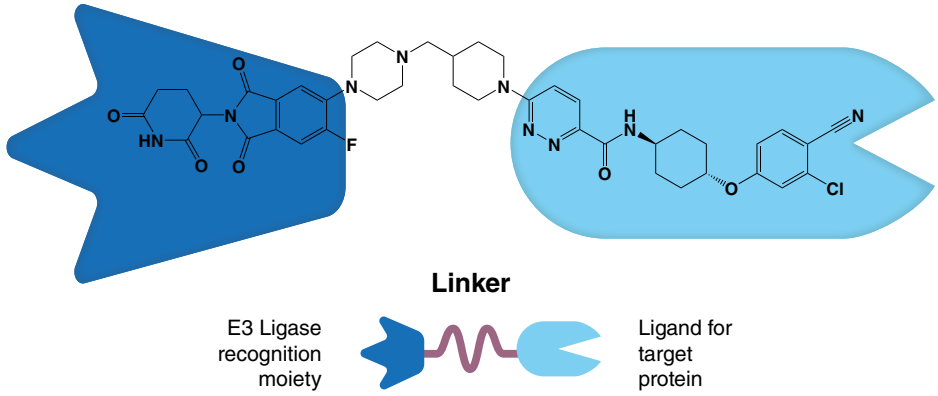


**Figure 1.1** Schematic of typical PROTAC structure.

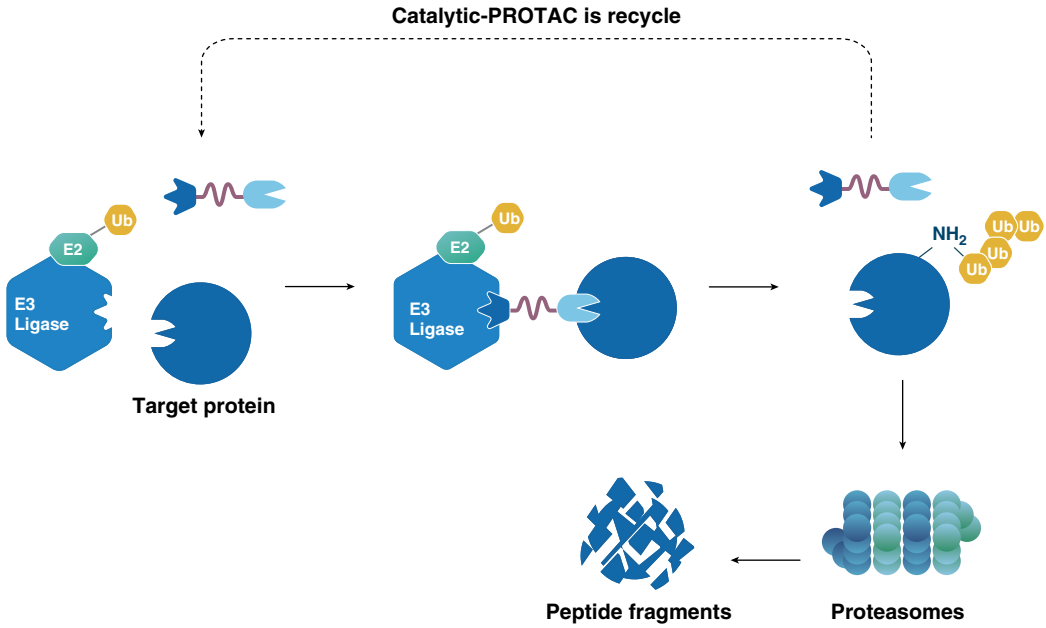


**Figure 1.2** Mechanism of action of PROTAC.

### 1.1.3 PROTAC Landscape Analysis

By using the event-driven pharmacology, PROTAC offers an attractive therapeutic concept to control target protein levels. Although this is a relatively new modality, it has made rapid progress in the drug discovery pipeline.

#### 1.1.3.1 PROTAC Research and Development Progress: International Pharmaceutical Companies

PROTAC has boomed in the last three years with Arvinas' two candidate molecules, ARV-110 and ARV-471, being the first to demonstrate positive clinical data. In addition, ARV-766, the company's

**Table 1.1** PROTAC research and development progress of some international pharmaceutical companies.

| Company | PROTAC | Indications | Target | Study phase |
|---|---|---|---|---|
| **Arvinas, Inc.** | ARV-471 | Breast cancer (ER+/HER2-breast cancer) | Estrogen receptor (ER) | Clinical phase III |
| | ARV-393 | B-Cell Malignancies | B-cell lymphoma 6 protein (BCL6) | Clinical phase I |
| | ARV-102 | Parkinson's Disease<br>Progressive Supranuclear Palsy | Leucine-rich repeat kinase 2 (LRRK2) | Clinical phase I |
| **Nurix Therapeutics, Inc.** | NX-2127 | B cell malignancies | BTK + IMiD activity | Clinical phase I |
| | NX-1607 | Immuno-oncology | CBL-B | Clinical phase I |
| | NX-5948 | B cell malignancies and autoimmune diseases | BTK | Clinical phase I |
| **Kymera Therapeutics, Inc.** | KT-474 | Allergic dermatitis, hidradenitis suppurativa, rheumatoid arthritis | Interleukin-1 receptor-associated kinase 4 (IRAK4) | Clinical phase II |
| | KT-621 | Immunology | STAT6 | Clinical phase I |
| | KT-333 | Solid tumors | STAT3 | Clinical phase I |
| | KT-253 | Solid tumors | MDM2 | Clinical phase I |
| **C4 Therapeutics, Inc.** | CFT7455 | Relapsed/refractory non-Hodgkin's lymphoma or multiple myeloma | IKZF1/3 | Clinical phase I/II |
| | CFT8919 | Non-small cell lung cancer (NSCLC) with drug-resistant EGFR mutation | EGFR L858R | Clinical phase I |
| | CFT1946 | V600 mutant cancers | BRAF V600 | Clinical phase I/II |
| **Bristol-Myers Squibb** | AR-LDD | Prostate cancer | AR | Clinical phase I |
| **Dialectic Therapeutics, Inc.** | DT-2216 | Tumor | BCL-XL | Clinical phase I |
| **Accutar Biotechnology Inc.** | AC0699 | Breast cancer | ER | Clinical phase I |
| | AC0682 | Breast cancer | ER | Clinical phase I |
| | Ac176 | Prostate cancer | AR | Clinical phase I |
| | AC0676 | Autoimmunity | BTK WT & C481S | Clinical phase I |

***Note:*** **The information was obtained from the official websites of the companies** mentioned in the table **and the deadline for information collection is** 15 **December 2024.**
**(Only research pipelines with compounds are listed.) Vividion Therapeutics, ERASCA, and other PROTAC companies do not disclose pipeline information.**

third PROTAC molecule, is set to enter the clinical phase. Other international companies, such as Kymera Therapeutics, C4 Therapeutics, and Nurix Therapeutics, that focus on developing protein degraders have a molecule in clinical phase I as well. In addition, other pharmaceutical companies such as Bristol-Myers Squibb, Dialectic Therapeutics, and Accutar Biotechnology also own a PROTAC molecule in clinical phase I (Table 1.1).

**Table 1.2** PROTAC R&D progress of Chinese pharmaceutical companies.

| Company | PROTAC | Indications | Target | Study phase |
|---|---|---|---|---|
| **Lynk Pharmaceuticals Co., Ltd.** | LNK01002 | Hematological tumor | — | Clinical phase I |
| **Kintor Pharmaceutical Limited** | GT20029 | Androgenetic alopecia, acne | AR | Clinical phase II |
| **Haisco Pharmaceutical Group Co., Ltd.** | HSK29116 | B-cell malignancies | BTK | Clinical phase I |
| **BeiGene Ltd.** | BGB-16673 | B-cell malignancies | BTK | Clinical phase I |
| **Cullgen Inc.** | CG001419 | Solid tumor | TRK | Clinical phase I/II |
| **Hinova Pharmaceuticals, Inc.** | HP518 | mCRPC for standard treatment failure | AR | Clinical phase I/II |
| | HP568 | Breast cancer | ER | Preclinical |
| | HC-X029 | Last-line treatment for mCRPC that has failed standard treatment | AR-sv | Preclinical |
| | HC-X035 | KRAS mutated cancers | Protein tyrosine phosphatase (SHP2) containing two SH2 (Src homology 2) domains | Preclinical |

***Note:*** **The information was obtained from the official websites of the above companies and the deadline for information collection is 15 December 2024.**
**Hangzhou Polymed Biopharma, Fendi Technology, Five Elements Biotechnology, Seed Therapeutics, and other PROTAC companies have not disclosed their pipeline information.**

#### 1.1.3.2 PROTAC Research and Development Progress: Chinese Pharmaceutical Companies

In China, PROTAC molecules are also developed by many companies, such as Lynk Pharmaceuticals, Kintor Pharmaceuticals, Haisco, Meizer Pharma, Cullgen, Hinova Pharmaceuticals, BeiGene, and Seed Therapeutics, a subsidiary of BeyondSpring, and all these companies have laid out in the PROTAC field. Among them, Lynk Pharmaceuticals, Kintor Pharmaceuticals, BeiGene, and Haisco own a PROTAC molecule in clinical phase I (Table 1.2).

### 1.1.4 Why Is DMPK Research Critical to PROTAC Drug Development?

Despite the popularity of PROTAC technology because of its unique mechanism of action, the development of PROTAC molecules still faces multiple challenges (Figure 1.3).

- Oral administration is the ideal drug delivery route for the treatment of most diseases, but because of its unique structure, PROTAC has a high molecular weight and poor solubility, which makes it difficult to meet the classical Lipinski's Rule of Five.
- It has poor *in vivo* and *in vitro* permeability, leading to poor absorption and low bioavailability.
- Several studies of PROTAC have shown that at appropriate or low concentrations, a normal ternary complex is formed. However, at high concentrations, a target protein-PROTAC or

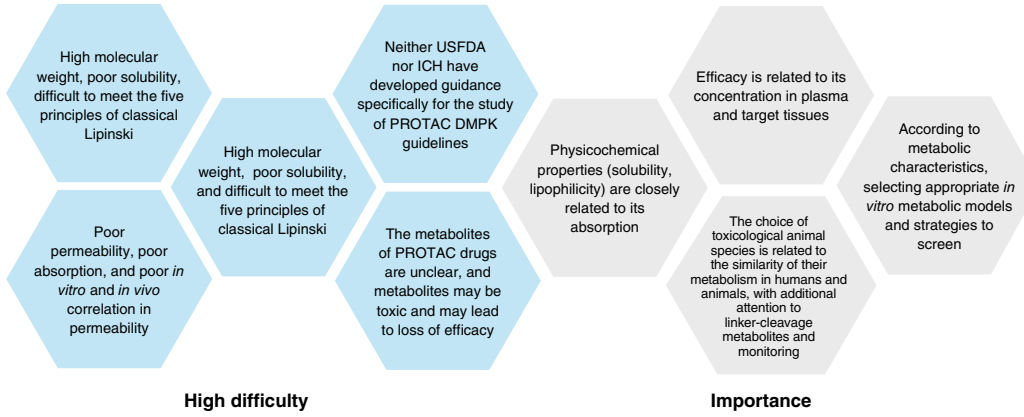


**Figure 1.3** Difficulties and importance of PROTAC research.

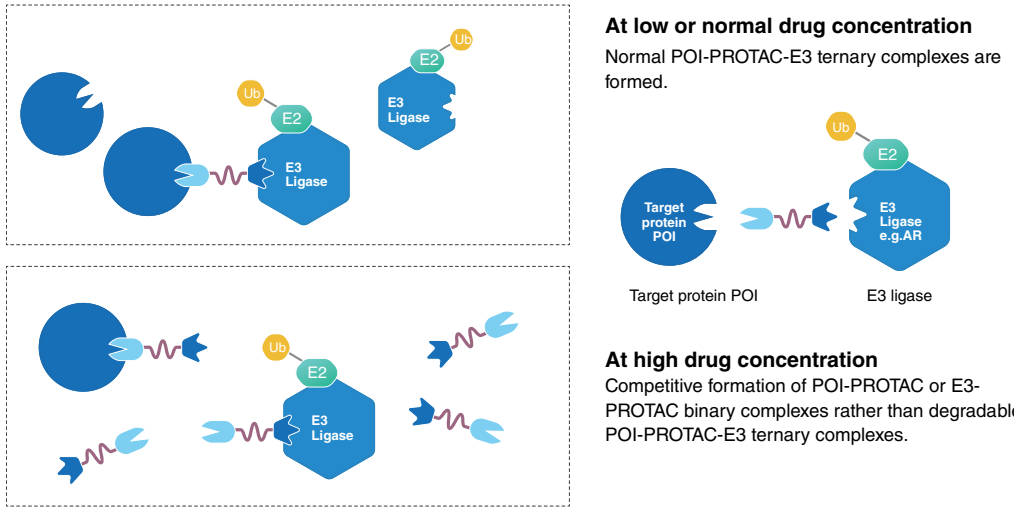


**Figure 1.4** Schematic representation of the hook effect.

E3 ligase-PROTAC binary complex is formed competitively, leading to reduced efficacy or toxic reactions, also known as the "hook effect" (Figure 1.4). Therefore, it is essential to fully understand the PKPD relationship of PROTAC, to timely modify the dosages.

- These molecules show high plasma protein binding in various species, and their plasma protein binding ratio may not be accurately measured when using the routine plasma protein binding evaluation method. Furthermore, the metabolism of PROTAC compounds in plasma might be affected by the high plasma protein binding.
- Metabolites of PROTACs may result in toxic reactions. Therefore, *in vivo* metabolite monitoring is a crucial part of its preclinical screening, with particular attention to linker-cleavage metabolites.
- There are no guidelines specifically for PROTAC drugs, hence, the guideline for small-molecule drugs is generally used as a reference.

### 1.1.5 The Strategy of the DMPK Study for PROTAC Drugs

The preclinical optimization of PROTAC drugs is mainly performed through the cascade optimization of physicochemical and DMPK properties [3].

- In the preliminary screening stage, the *in vivo* and *in vitro* characterization of PROTAC molecules is required, including physicochemical properties, permeability, protein binding, and drug-drug interaction (DDI).
- In the optimization stage, to better understand the DMPK properties of PROTAC, such as absorption and metabolism, the focus should be on improving the metabolic clearance and solubility of PROTAC molecules combined with the PK properties of extravascular drug delivery,. The structure of PROTACs poses a challenge in improving their permeability. Therefore, at this phase, more attention should be paid to solubility and metabolic stability when administered orally.
- At the PCC stage, in to order to obtain a more in-depth exposure–response relationship, PROTAC molecules with potent and better oral bioavailability can be used in further pharmacokinetics/pharmacodynamics (PK/PD) studies.

### 1.1.6 Summary

Although there is no marketed drug for PROTAC until July 2024, it is attracting more and more biopharmaceutical innovators and entrepreneurs to compete on this brand-new track. Over the course of more than 20 years of development, PROTAC has redefined small molecules with its unique mode of action. It has shattered the conventional rules of drug discovery and ushered in a new era of drug development. We anticipate a growing number of designed and developed PROTAC molecules, which will unlock fresh opportunities in various disease areas and give hope to a greater number of patients.

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## 1.2 Strategies to Improve the Oral Bioavailability of PROTACs

*Liping Ma, Jie Hu, Chengyuan Li, Jing Jin*

### 1.2.1 Introduction

Because of its unique mechanism of action, PROTAC has gradually become one of the most popular small molecules over the last five years. However, there are many obstacles to overcome due to its druggability. One of the most discussed challenges is that, unlike traditional small-molecule drugs, PROTAC does not adhere to the classical Lipinski's Rule of Five. PROTAC is considered to violate the tenets of medicinal chemistry and functions as a redefined small-molecule compound. Consequently, poor oral absorption remains a common issue for PROTAC compounds. Here, we discuss the PROTAC molecules' properties that are closely related to oral absorption and bioavailability, including solubility, lipophilicity, permeability, metabolic stability, etc., to provide ideas for further research and development of PROTAC molecules.

### 1.2.2 PROTAC Molecules Hardly Meet Lipinski's Rule of Five

Oral small-molecule drugs with high bioavailability and desired pharmacokinetic properties generally meet Lipinski's Rule of Five:

a) The molecular weight is less than 500.
b) No more than 5 hydrogen bond donors.
c) No more than 10 hydrogen bond acceptors.
d) Octanol–water partition coefficient log P not greater than 5.
e) No more than 10 rotatable bonds.

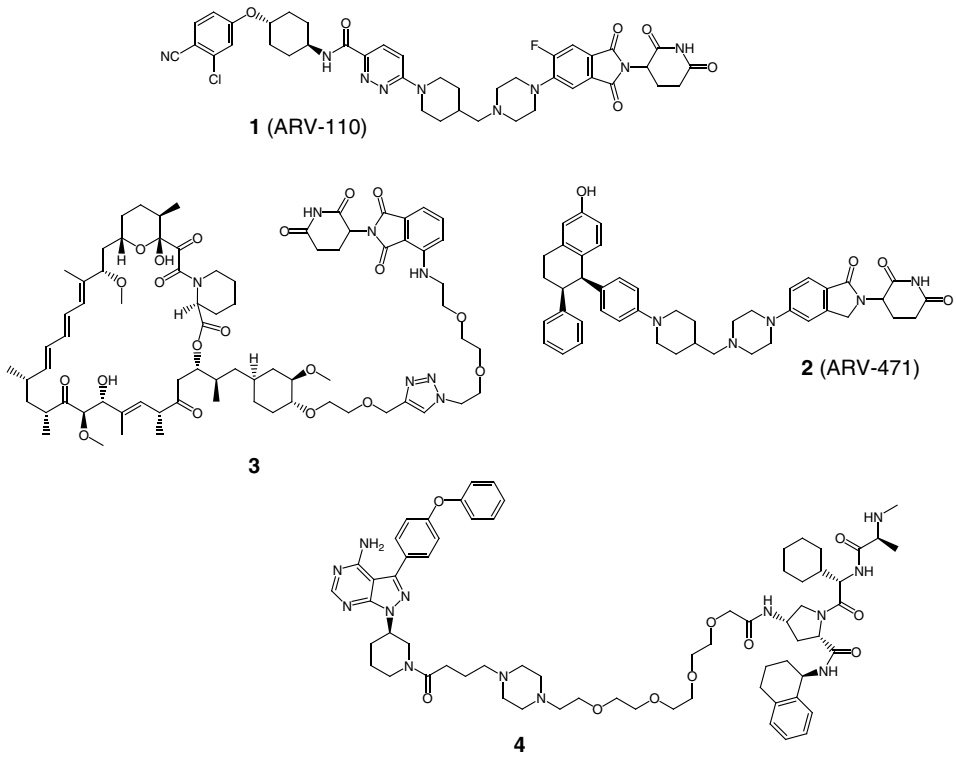
In terms of molecular weight, PROTAC consists of three key structural parts, a ligand binding with the target protein, a ligand binding with the E3 ubiquitin ligases, and a linker connecting the two ligands, which results in a large molecular weight of PROTAC, typically more than 700 Da [1]. PROTAC is also poorly soluble and permeable.

The two PROTAC molecules, ARV-110 and ARV-471, which have entered Phase II clinical trials, exhibit physical and chemical characteristics that are closer to Lipinski's Rule of Five (Figure 1.5). This may indicate that PROTACs that adhere more closely to Lipinski's Rule of Five may possess better pharmacokinetic properties [2].

### 1.2.3 Strategies to Improve PROTAC's Bioavailability

#### 1.2.3.1 Improve the PROTAC's Solubility

The solubility of compounds in a pH 7.4 phosphate buffer solution (PBS) is typically measured by conventional solubility studies. However, since drug absorption occurs in the gastrointestinal tract, it is important to consider the solubility of PROTAC in physiological media as well. It has been reported that the solubility of PROTAC molecules significantly improves in fasted-state simulated intestinal fluid (FaSSIF) and fed-state simulating intestinal fluid (FeSSIF), with optimal solubility observed in FeSSIF (Figure 1.6). The clinical trial design of ARV-110 and ARV-471 published by Arvinas disclosed that the phase I clinical administration modes of these two PROTAC molecules




| | MW (Da) | cLogP | HBD | HBA | TPSA | NRotB |
|---|---|---|---|---|---|---|
| bRo5 outer | 1000 | 10 | 6 | 15 | 250 | 20 |
| 1 | 812 | 4.5 | 2 | 10 | 181 | 11 |
| 2 | 724 | 6.0 | 2 | 5 | 96 | 7 |
| 3 | 1426 | 5.3 | 4 | 21 | 338 | 23 |
| 4 | 1241 | 3.5 | 5 | 15 | 262 | 35 |

**Figure 1.5** Structure and physicochemical properties of ARV-110, ARV-471, and two other PROTAC molecules. *Source:* [2] / with permission of Taylor & Francis Group.

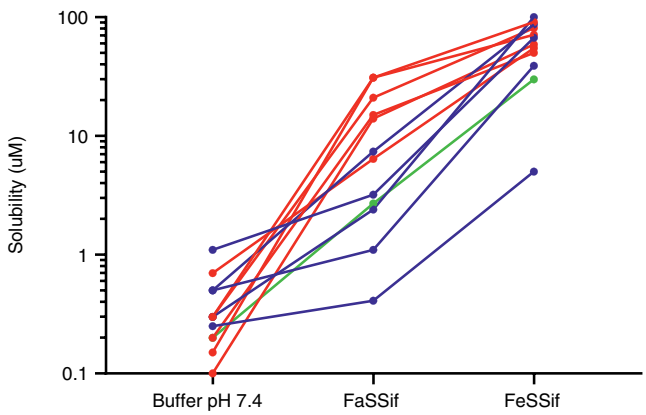



**Figure 1.6** Solubility of PROTAC molecules in different buffer solutions. *Source:* [3] / ELSEVIER.

are both "once daily with food." Our study also found that postprandial administration increased the exposure of some PROTAC molecules in animals. This suggests that the *in vivo* pharmacokinetics of PROTAC may improve its *in vivo* drug exposure via postprandial administration.

Another way to solve the solubility problem of the PROTAC is to use optimized formulations. Studies have shown that the in vivo exposure can be increased by employing amorphous solid dispersions (such spray drying and hot melt extrusion), nano delivery systems, self-emulsifying delivery methods, or different solvents to make the formulation clear.

#### 1.2.3.2 Improve the PROTAC's Permeability

Permeability is another crucial factor that affects oral bioavailability. On the one hand, drugs need to pass through the membrane barrier of small intestinal epithelial cells for oral absorption. On the other hand, for intracellular protein degradation, PROTACs also need to enter target cells. It has been demonstrated that the cell permeability of PROTACs can be significantly increased by substituting the PEG linker with a 1,4-disubstituted benzene ring [4].

In the preclinical compound screening stage, it is critical to select an appropriate *in vitro* permeability model for PROTAC. Conventional models for *in vitro* permeation research include Caco-2, MDCK, LLC-PK1, and PAMPA. When researching PROTAC molecules, PAMPA is not recommended as a suitable *in vitro* permeability model because of the limitations of its noncellular structure. Caco-2, MDR1-MDCK, or LLC-PK1 cells can be used to evaluate the *in vitro* permeability of PROTAC, whereas PROTAC molecules showed low permeability in most of the models (Figure 1.7a–d).

In addition to low permeability, the low solubility and high nonspecific binding (NSB) would also lead to the insufficient recovery of PROTAC molecules in permeability models. Low recovery makes it hard to determine whether the permeability data are accurate, thus greatly reducing the credibility of the data. We carried out additional validation studies to solve this problem. The recovery of PROTAC molecules in the Caco-2 cell models can be greatly improved by using a transfer buffer with BSA or a physiological medium as the transfer buffer. The optimized Caco-2 cell systems have been recommended for *in vitro* permeability evaluations of PROTAC.

#### 1.2.3.3 Improve the PROTAC's Metabolic Stability

When absorbed by the intestine, the compound undergoes metabolism by the liver or intestine before it enters the circulatory system. This process is known as "first-pass" metabolism, which restricts the oral absorption of many drugs. One strategy to enhance the oral bioavailability of PROTACs is to improve their metabolic stability and reduce the first-pass metabolism. Various approaches have been explored to achieve this, including altering the length of the linker, modifying the anchor point of the linker, employing cyclic linkers, and changing the attachment site of the linker [5].

#### 1.2.3.4 Choose Smaller E3 Ligand

PROTACs' properties are closely related to the type of E3 ligase ligands. The most commonly utilized E3 ligases for PROTAC mainly include CRBN, VHL, cIAP, and MDM2, with CRBN and VHL being the most frequently employed. As shown in Figure 1.8, CRBN has a smaller volume compared to VHL ligands. It has been reported that VHL ligand-containing PROTACs usually have poor oral absorption due to larger molecular weights [2]. Vasanthanathan et al. compared the chemical properties of PROTAC molecules based on different E3 ligases with oral drugs included in Drugbank and found that the PROTAC based on VHL **(green)** is far away from the oral drug region **(blue)** and it has been reported that the bioavailability of such PROTAC is indeed low. In contrast, the PROTAC based on CRBN E3 ligase **(red)** is closer to the oral drug region [2]. The two PROTAC molecules (ARV-110 and ARV-471) that have entered the clinical phase II are with CRBN E3 ligase. Therefore, searching for new E3 ligands with smaller molecular weights is worth exploring.

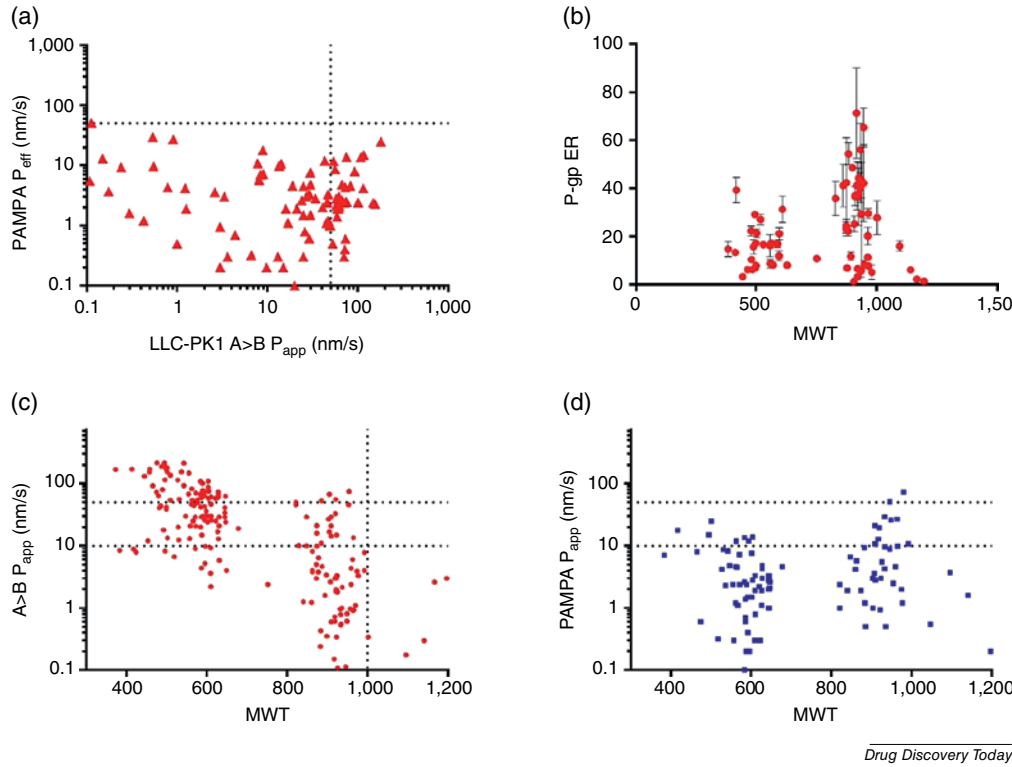



**Figure 1.7** Molecular weight and permeability of PROTAC. *Source:* [1] / ELSEVIER. (a) The correlation between PAMPA and LLC-PK1, the area under the dotted line represents low permeability; (b) relations between P-gp efflux ratio and molecular weight; (c) the permeability of compounds with different molecular weights in LLC-PK1 cell model, molecular weight less than 650 is a single ligand, and molecular weight more than 650 is a complete PROTAC; (d) the permeability of compounds with different molecular weights in the PAMPA model, molecular weight less than 650 is an individual ligand, and molecular weight more than 650 is an intact PROTAC.

#### 1.2.3.5 Introduce Intramolecular Hydrogen Bonds

PROTACs usually have high polarity and many rotatable bonds, and these structures generally make it difficult to penetrate the lipid bilayer of the cell membrane. Recent research found that the formation of intramolecular hydrogen bonds can reduce the polar molecular surface area of PROTACs and then improve their permeability. Under the action of intramolecular hydrogen bonds, an original strip-type molecule will be transformed into a "ball" form, which makes it easier to penetrate the lipid bilayer of the cell membrane [6].

#### 1.2.3.6 Select Appropriate Solutions

PROTAC compounds generally have long structures, large molecular weight, high polarity, and a large number of rotatable bonds. Atilaw et al. showed that a PROTAC acts like a chameleon in that its conformation changes with the environment [6]. In solutions that mimic extra- (dimethyl sulfoxide, DMSO) or intracellular (DMSO mixed with water by 10:1), PROTAC molecules present an elongated shape and have a high molecular polarity. However, in the solution that mimics a cell membrane interior (chloroform), PROTAC molecules are folded by forming intramolecular hydrogen bonds and $\pi-\pi$ interaction, thus becoming a molecule with a smaller polar surface area (Figure 1.9). In other words, it shifts from the conformation of a polar molecule to the conformation of a nonpolar molecule. This finding suggests that the permeability of PROTAC may be related to whether it can form a conformation with a small polar surface area during the permeation process.

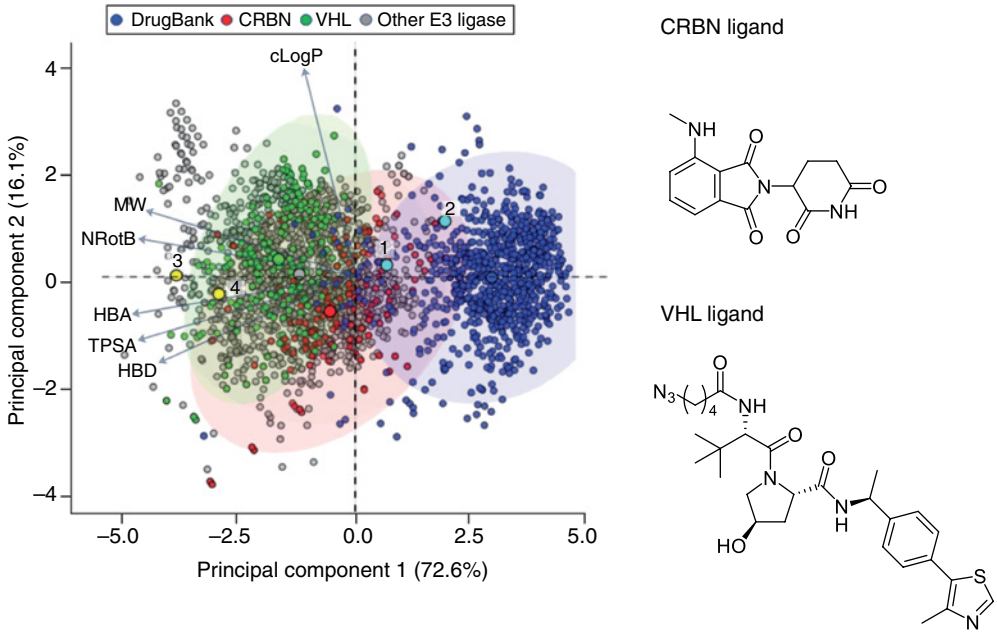



**Figure 1.8** Structures of CRBN, VHL ligands, and comparison of chemical properties of PROTAC molecules with oral drugs included in the Drugbank. Blue circles represent oral drugs included in the Drugbank ($n$ = 888), and red, green, and grey circles represent PROTAC of CRBN, VHL, and other E3 ligases ($n$ = 2,082), respectively. *Source:* [2] / with permission of Taylor & Francis Group.

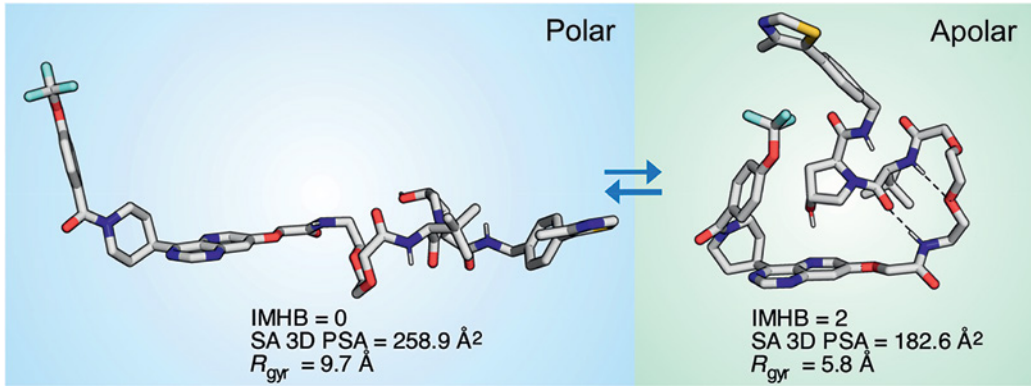



**Figure 1.9** Conformation change of PROTAC in different solutions. *Source:* [6] / American Chemical Society.

#### 1.2.3.7 Use Prodrug Strategy

Prodrug is a common approach to improving the oral bioavailability of drugs. A prodrug is obtained by structural modification of pharmacologically active compounds. Prodrugs themselves have little or no activity, and pharmacologically active parent drugs will be released *in vivo* by enzymatic catalysis. Chemists designed a prodrug from a PROTAC by adding a lipophilic group to the CRBN ligand. The results showed that the bioavailability of a PROTAC was significantly increased by prodrug design [7]. To improve the oral bioavailability of different PROTACs with similar E3 ligands, CRBN ligand-based prodrug design can also be used (Figure 1.10). However, increasing the molecular weights of PROTACs is a potential problem when designing prodrugs for PROTACs.

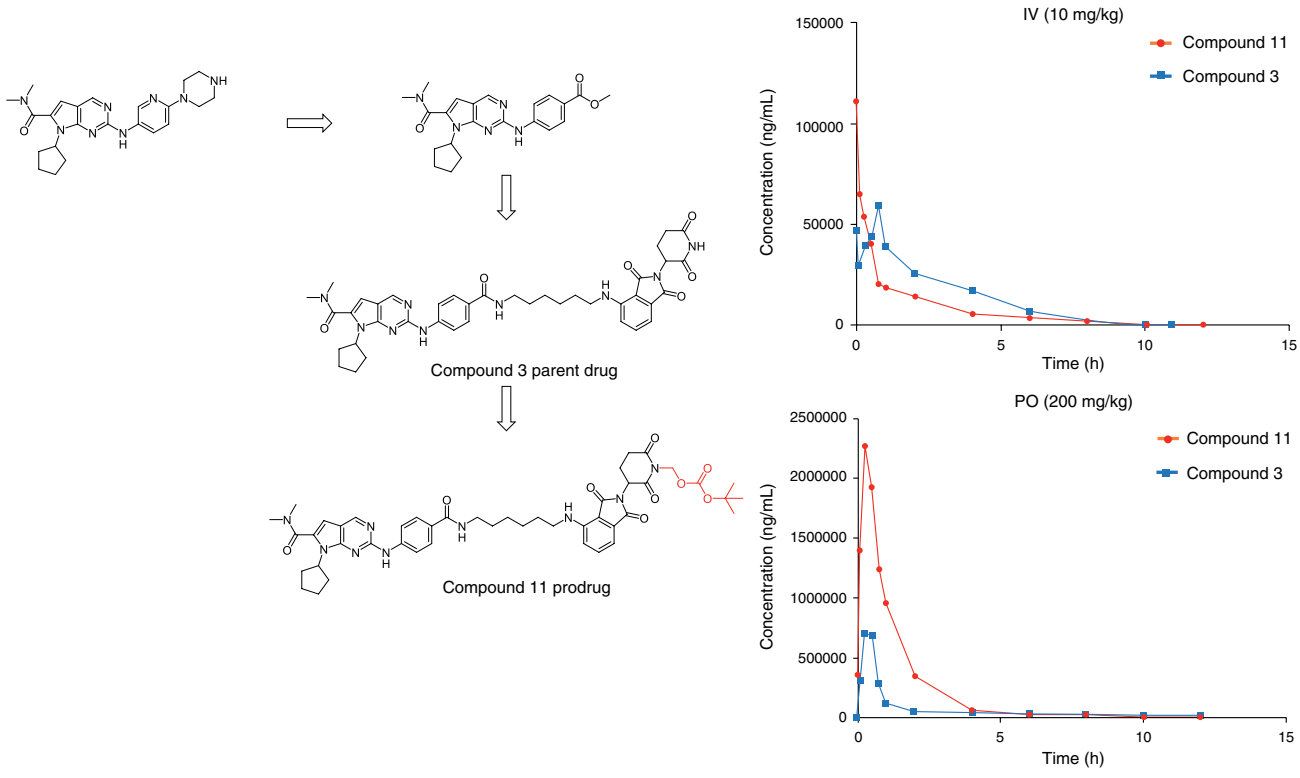



**Figure 1.10** Oral bioavailability of compound 3 was significantly improved after its prodrug design as compound 11. *Source:* [7] / ELSEVIER.