Physicochemical and Biomimetic Properties in Drug Discovery

Chromatographic Techniques for Lead Optimization

Klara Valko

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PREFACE

During the past 10 years I have been teaching the Physchem/ADME (absorption, distribution, metabolism, and elimination) module for "Drug Discovery M.Sc." students at the School of Pharmacy, University of London (University College London since 2012). The module covers ADME and the underlying physicochemical properties of drugs. This book is intended to summarize the course material, providing detailed explanations of the physicochemical aspects of drug absorption and distribution *in vivo*. It is well recognized now that drug molecules occupy a relatively small physicochemical property space in comparison to the huge number of possible physicochemical entities. Lipophilicity, solubility, permeability, and the charge state of molecules are the most important properties that influence absorption and *in vivo* distribution. Thus, the measurement and calculation of lipophilicity, solubility, permeability, and the charge state are essential early on in the drug discovery process in order to select compounds for further studies. Compounds selected in this way have the best possible chance to make it to development and eventually help patients recover or at least improve their quality of life. The principles of the measurement of physicochemical and biomimetic properties are explained in more detail. Special emphasis is given to the chromatographic measurements of various physicochemical and biomimetic properties of drugs. It will be shown how to interpret the chromatographic retention data by putting them into various models for the estimation of *in vivo* distribution behavior of compounds. Other techniques are also mentioned, and further references are provided but not discussed in detail.

The chromatographic dynamic equilibrium process provides an excellent model to describe a compound's *in vivo* distribution between the moving plasma/blood compartment and the stationary tissue compartments. The reader will learn that chromatography is a powerful technique not only for the separation of closely related compounds but it is also very useful to measure a compound's interactions with various biomimetic stationary phases covered with alkyl chain, proteins, and phospholipids. The obtained binding data are suitable for deriving quantitative structure–physicochemical property relationships (QSPR) that can be used in drug design. Mathematical models can be constructed for the estimation of a compound's *in vivo* distribution. Active transporters and other specific binding of the

molecules to various types of proteins and phospholipids can, of course, modulate the estimated distribution, and these potential interactions need to be taken into account during the lead optimization process. When significant differences are observed between the *in vivo* measured and estimated distribution behavior of a compound based on physicochemical properties, it indicates to the drug discovery scientist that the compound undergoes some active transport mechanism. The active transport process can push the compound concentration from the thermodynamic equilibrium, but it requires constant energy investment from the body via biochemical processes.

This book contains previously published ideas, concepts, and methodologies. This literature review is far from comprehensive; it is rather critical and selective in order to reveal various ways to approach problems. However, the book provides simple descriptions and explanations of the essence of selected publications that usually help students understand the methods, results, and conclusions of scientific papers. A chapter dedicated to chromatography describes the basic principles and practical considerations that are needed to set up and run the measurements that are discussed in later chapters. Examples are shown how to use experimental data in various models of absorption, tissue binding, volume of distribution, and other *in vivo* ADME characteristics of the drugs. The property data obtained by fully automated chromatographic measurements can be used to select and prioritize compounds for further *in vitro* and *in vivo* studies at early stages of the drug discovery process. A special chapter is dedicated for the interpretation of the data, the interrelationship between the physicochemical properties, and some structure–property relationships in the hope that medicinal chemists can use the information for designing new potential drug molecules.

Although there are several books that cover similar topics, this book is unique in many ways. First, the chromatographic technique is normally described as an analytical separation method, and it is rarely discussed as a tool for property measurements of drug discovery compounds. There are hundreds of research papers and a few reviews that demonstrate the usefulness of the technique for such purposes but the general explanations together with a deeper understanding has not been published in a textbook yet. Sufficient detail is provided to enable the reproduction of chromatographic measurements in any laboratory equipped with HPLC. Second, this book is unique in the sense that it contains some new insight into the interdisciplinary knowledge needed for designing efficient drugs with minimal side effects. The intention is to reveal the relationships between various disciplines such as physical chemistry, analytical chemistry, biology, and pharmacokinetics/pharmacodynamics by the underlying basic thermodynamic laws.

Communication between scientists from different disciplines is hindered not only by differences in vocabulary but also many times by the use of different units. For example, the first measurements of activity of a compound is usually expressed as a pIC_{50}, which means a quantity of the compound expressed as the negative logarithm of the molar concentration that causes 50% of the maximum inhibition on a particular target or enzyme. Then we measure the solubility in millimolar or

milligram per milliliter units as the maximum soluble concentration of the drug. Experts in drug metabolism and pharmacokinetic (DMPK) measure the quantity of drug absorbed as a percentage, the plasma protein binding is measured as unbound fraction, and volume of distribution as liters per kilogram. Finally, the patient takes a quantity of drug (dose) expressed as an *X* mg tablets three times a day. In all of the above instances, we would like to express the amount or concentration of drug molecules. Using different units in various phases of the drug discovery may hinder our understanding. I do not think we can change this practice in the near future. However, at present, we can learn to convert the units and understand the relationships between various measurements. The mathematical and physical–chemical rules serve as links between disciplines in this book. It is demonstrated that mathematical approaches (paying special attention to the units of the measurements) help interdisciplinary thinking, converting, and translating knowledge between scientists working in different fields. In this way, we can maximize our understanding of the huge amount of data that are normally generated during the drug discovery process.

Chapter 12 presents examples, mostly using historical data of successful known drug molecules or published project examples from several pharmaceutical companies. At the end of each chapter, there is a short summary containing the conclusions and help revision for students taking examinations of this material. Finally, some typical examination questions are added at the end of each chapter to be able to test the reader's comprehension of the material. The answers are provided in Appendix A.

Some of the conclusions have been made on the basis of the author's personal view and experience in the subject. These do not always agree with the accepted and widely used theories in big pharmaceutical companies. Ideas for further research areas, unanswered questions, and hypotheses are presented *in italics* in order to encourage and motivate readers to form their own opinion. The book contains several questions that were raised by the students during the lessons and the answers still have to be found by the next generation of scientists. Therefore, I hope that the text raises the motivation and enthusiasm of scientists who are willing to engage themselves in drug discovery for a long period.

This book provides the essential experimental details for the determination of physicochemical and biomimetic properties that can be carried out in any analytical laboratories equipped with HPLC. Medicinal chemists will be able to understand these properties and the structure–property relationships described here and successfully use them in drug design and lead optimization. DMPK and ADME scientists will be able to interpret the results of their *in vitro* and *in vivo* experiments after thorough comprehension of the content, and it will help them in designing the necessary experiments to support the drug discovery process. I am aware that several drug discovery institutions and universities have already set up the chromatography-based determination of lipophilicity, protein binding, and phospholipid binding. I hope that with the help of this book, they will be able to use these properties in their full potential for the estimation of *in vivo* properties, thus reducing the need for animal experiments.

Finally, I would like to thank all those who have been of tremendous help during the preparation of this book. I am grateful to my past and present colleagues at GlaxoSmithKline, especially to Derek Reynolds, Chris Bevan, and Alan Hill with whom I gained experience in the field of physicochemistry and its application in drug discovery and who have supported me in many ways. I would like to thank my colleagues Shenaz Bunally, Elisabetta Chiarparin, and Paul Leeson, who read the manuscript and gave me excellent advice to improve it. I would like to acknowledge the help I have received from the colleagues at the School of Pharmacy, University College London, Professor David Thurston, Professor Simon Gibbons, Professor Anne Stevenson, Dr. Michael Munday, Dr. Mire Zloh, and Dr. Rosemary Smyth.

I have received the greatest inspiration for the preparation of this book from my students. Many of them are extremely talented, providing me with the hope that they will continue to improve drug discovery and will help millions of patients who desperately need cure for their disease. Drug Discovery M.Sc. students from year 2011 to 2012 have gone through the manuscript and provided excellent feedback where I need to explain the material in more detail or more clearly. I would like to thank especially Godfrey Mayoka, Samar Youshif, and Hajir Azam for carefully reading the manuscript. I have used and referenced the project works of Manju Kalyani and Andriana Rapti, who carefully investigated and validated the methodology for solubility determination and α-1-acid glycoprotein binding measurements during their M.Sc. studies.

I am very grateful for the well-known experts who provided valuable suggestions and corrections for specific chapters. Professor Krisztina Takács-Novák, School of Pharmacy, Semmelweis University, has reviewed chapters on solubility and pK_a; Dr. Alex Avdeef, In Adme, Ltd., has reviewed the chapter on permeability; Professor Michael Abraham, University College London; has reviewed the chapter on lipophilicity. They are recognized experts in these fields and their valuable corrections and suggestions are appreciated.

I would like to thank my family and friends for their emotional support and understanding, especially to my son Adam Valko who coped very well with his studies and his life on his own during the preparation of this book.

CONTENTS

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THE DRUG DISCOVERY PROCESS

The way we discover drugs now is very different from the process followed 30–50 years ago [1–3]. In the past, the drug discovery process was based on the structure of known active compounds. The structure of the active compound, which was either endogenous (e.g., acetylcholine, adrenaline, and steroid hormones) or a natural product (e.g., morphine, papaverine, cocaine, atropine, and digitalis glycosides), was identified and modified by a trial-and-error method. Scientists isolated the active material, revealed the structure and the mechanism of the pharmacological action, and based on this knowledge they would modify the chemical structures with the aim of improving the activity, decreasing the toxicity, and increasing the duration of action. Using this approach, they discovered, for example, a series of cholinesterase inhibitors, a series of adrenaline analogs for sympatomimetic or sympatolytic compounds, semisynthetic steroid hormones, and many more. Another example is pethidine containing the pharmacophores of morphine. The ethoxy derivative of papaverine called *No-Spa* has a longer half-life and is a stronger spasmolytic agent. Figure 1.1 shows a few analog-based drug molecules designed from natural compounds. There are numerous examples of naturally occurring molecules or their derivatives being used as drugs, such as warfarin (anticoagulant derived from dicumarol found in sweet clover, hirudin (anticoagulant from leeches), statins (a fungal metabolite that reduces plasma

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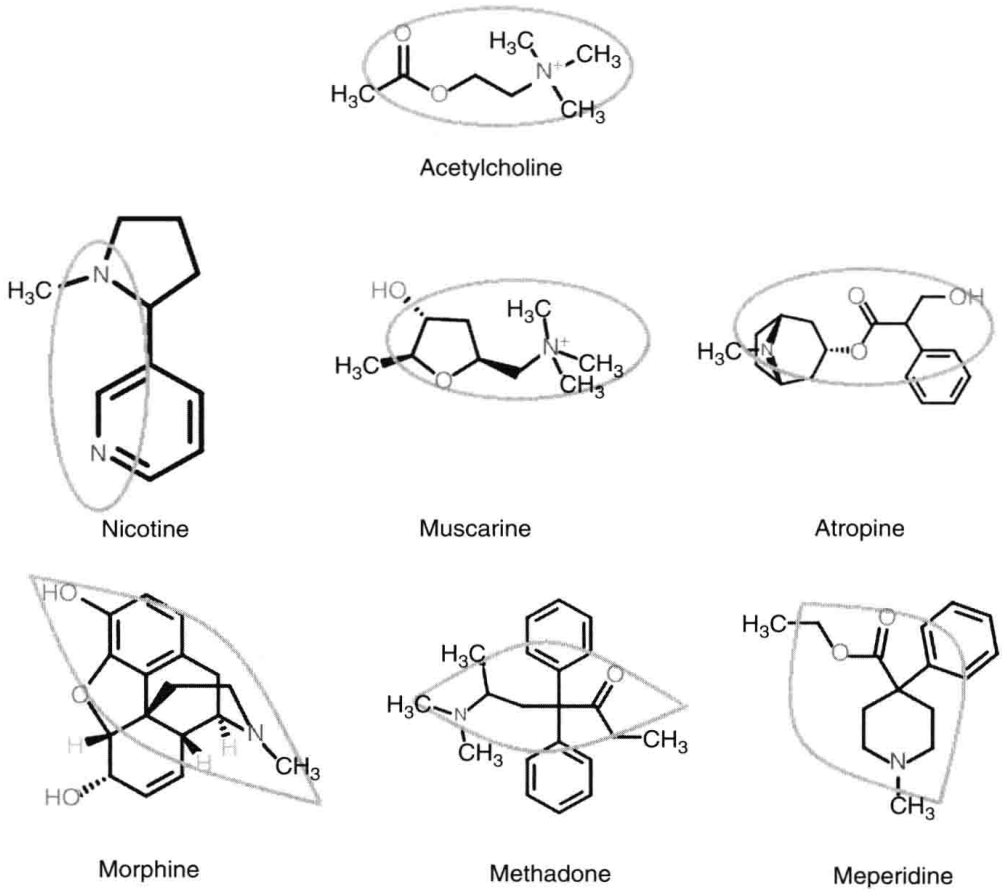

Figure 1.1 Drug molecules designed as analogs of natural active molecules containing the active pharmacophore.

cholesterol), vinca alkaloids (anticancer drugs isolated from periwinkle plant families), and antibiotics (derived from fungal metabolites).

Today, drug discovery utilizes recent advances in biochemistry and genetics. Drug discovery usually starts with discovering a so-called "target" that can be enzymes or receptors, such as G protein-coupled receptors (GPCR) and enzyme targets, such as kinases, ion channels, and hormone receptors.

The medicinal chemists and computational chemists work closely together to generate ideas about potential active molecules that would fit to the "target" molecule. They develop a so-called high throughput screening (HTS) method that enables the big pharmaceutical companies to screen large number of compounds against a particular target. Compounds that show activity in HTS are called *hits*. The activity of the "hits" is further evaluated by repeated experiments for obtaining the standard concentration–potency curve. Figure 1.2 shows a typical concentration–response/potency profile during hit generation and confirmation.

This phase of the drug discovery process is often called *hit generation*. Synthetic chemists then synthesize a whole library of molecules, making numerous