Pharmacophores and Pharmacophore Searches

Edited by
Thierry Langer and Rémy D. Hoffmann

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and Rémy D. Hoffmann

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Preface

The idea is very straightforward: find and define all locations in space at a certain time of all substituents of a bioactive molecule that contribute to its biological activity. The readout would be a three-dimensional map – with respect to structure – that represents a minimal set of substituents which would adapt to a negative casting mold of the target binding site. By estimating or calculating the electronic and geometric properties of the substituents at their locations you would expand the 3D map to multiple dimensions. You call it a pharmacophore. After that, theoretically, you would walk through the Periodic Table and create a set of substituents, tied together by an appropriate backbone to fulfill all electronic and steric requirements of the pharmacophore. Finally, you obtain a new chemical entity with good prospects for activity at the target of choice.

But you get more. A "map" is a tool that relates objects to each other. These relations may be distances as they appear on a roadmap, it may be frequencies or densities on a web exploration map or it may be metabolism–emotion relationships in a brain map. Hence the pharmacophoric map can be used as a filter by matching the property vectors and a library of synthetic and/or virtual ligands, sorting out putative binders.

Well, "*Before the gates of excellence the high gods have placed sweat; long is the road thereto and rough and steep at first*" (Hesiod, *Work and Days*).

In the present book, Thierry Langer and Rémy Hoffmann give us a description of the long road with a firm sight on what can be done now and what is still to be achieved. Camille Wermuth, a doyen of the field, starts the arc of contributions shaping the history of the pharmacophore concept. The subsequent chapters are grouped into two major parts: "Pharmacophore Approaches" and "Pharmacophores for Hit Identification and Lead Profiling: Applications and Validation". Much attention is devoted to the problem of alignment and cost of energy. The contributions face the problems not only from the small molecule, the ligand's view, but also from the complementary side, the receptor's binding site. Experience from both industrial research and development laboratories and academic research is covered, especially in the applications and validation part, which gives the reader a feeling for the feasibility and implementation of the approaches and bridges the gap between theory and practice.

The series editors are indebted to the authors and the editors who devoted much of their time to educational purposes and rendered this exciting issue possible.

We also want to express our gratitude to Renate Doetzer and Frank Weinreich of Wiley-VCH for cooperative and easy collaboration and their invaluable support in this project.

April 2006

Hugo Kubinyi, Weisenheim am Sand
Gerd Folkers, Zürich
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A Personal Foreword

Pharmacophores! Behind this simple word and concept that may be seen somehow reductionist, a vast amount of information about bioactive molecules and their structure–activity relationships is hidden, but available. Both of us had the privilege of having been exposed first to these important tools in medicinal chemistry by Professor Camille-Georges Wermuth some 20 years ago at the faculty of Pharmacy of the Université Louis Pasteur in Strasbourg. In this academic laboratory, several drug molecules have been developed that were successfully brought to the market. The pharmacophore concept was used always keeping in mind the need to understand, explain and predict molecular interactions with the targets in addition to structure–activity relationships. Its practical applicability for medicinal chemists made it an excellent communication tool between modelers and synthetic chemists. We are therefore grateful to Professor Wermuth, who has kindly accepted to write the first chapter of this book.

Since that time, we have been working in the context of using and developing tools and methods for rational molecular design, in both academic and industrial environments. We have seen several key changes in paradigms, such as combinatorial chemistry and associated HTS techniques, structure-based design strongly related to the ever-increasing number of characterized 3D structures of target proteins and the emerging virtual screening technologies. Pharmacophores have somehow been neglected in the last decade, although some gold standard tools were already available to the research community that have unfortunately not been further developed. However, as the hype about both structure-based design and large-scale HTS has flattened, a new area for pharmacophore tools obviously has begun.

As outlined in this book, several innovative tools and approaches for pharmacophore-based modeling and screening have emerged recently in the literature. Since the last textbook on pharmacophores and their usage in drug discovery, edited by Osman F. Güner in 2000, considerable progress has been achieved and also a large number of success stories in different application areas have clearly demonstrated the power of this approach. We felt that now was the right time to summarize these developments and their applicability. Therefore, we are grateful to the series editors, Professors Hugo Kubinyi, Gerd Folkers and Raimund Mannhold, for having invited us to edit a book focusing on this exciting research area. Starting with an introductory historical overview, ligand-based

approaches, including 3D pharmacophores and 4D QSAR, are discussed, and also the concept and application of pseudoreceptors. Another section on structure-based approaches includes pharmacophores from ligand–protein complexes, FLIP and a chapter on 3D protein-ligand binding interactions. The whole is rounded off with a complete section devoted to applications and examples, including modeling of ADME properties.

The intention of this book is to provide the reader with the different aspects of pharmacophores and pharmacophore-based screening in the drug discovery and development context. Each chapter is written by well-recognized experts in their respective fields. We take the opportunity to thank them all for their contributions to this book. It was a privilege to interact with them in order to bring this ambitious project to fruition. We hope that this book will contribute to stimulating further developments in this area, since we feel that there is still room for new technologies and improvements around pharmacophores. Happy reading!

Innsbruck and Paris, March 2006

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Part I
Introduction

1
Pharmacophores: Historical Perspective and Viewpoint from a Medicinal Chemist

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Since the appearance of computer-aided structure–activity studies, the term "pharmacophore" has become one of the most popular words in medicinal chemistry. However, depending on their scientific background and/or traditions, the different medicinal chemistry groups attribute various meanings to this term. Therefore, it appeared necessary to devote a brief paragraph to the definition of the word pharmacophore, and this is followed by a historical perspective and finally by some comments from a medicinal chemistry practitioner.

1.1
Definitions

Many authors use the term "pharmacophores" to define functional or structural elements possessing biological activity. This does not correspond to the official definition elaborated by an IUPAC working party and published in 1998 [1]: *A pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response*. As a consequence:

1. The pharmacophore describes the essential, steric and electronic, function-determining points necessary for an optimal interaction with a relevant pharmacological target.
2. The pharmacophore does not represent a real molecule or a real association of functional groups, but a purely abstract concept that accounts for the common molecular interaction capacities of a group of compounds towards their target structure.
3. Pharmacophores are not specific functional groups (e.g. sulfonamides) or "pieces of molecules" (e.g. dihydropyridines, arylpiperazines).

A pharmacophore can be considered as the highest common denominator of a group of molecules exhibiting a similar pharmacological profile and which are recognized by the same site of the target protein. However, despite the official

definition and the remarks made above, many medicinal chemists continue to call pharmacophores some specific functional groups, especially if they appear to be often associated with biological activity.

1.1.1
Functional Groups Considered as Pharmacophores: the Privileged Structure Concept

The retrospective analysis of the chemical structures of the various drugs used in medicine led medicinal chemists to identify some molecular motifs that are associated with high biological activity more frequently than other structures. Such molecular motifs were called privileged structures by Evans et al. [2], to represent substructures that confer activity to two or more different receptors. The implication was that the privileged structure provides the scaffold and that the substitutions on it provide the specificity for a particular receptor. Two monographs deal with the privileged structure concept [3, 4].

Among the most popular privileged structures, historical representatives are arylethylamines (including indolylethylamines), diphenylmethane derivatives, tricyclic psychotropics and sulfonamides. Dihydropyridines [5], benzodiazepines, [2, 5], *N*-arylpiperazines, biphenyls and pyridazines [6] are more recent contributions.

A statistical analysis of NMR-derived binding data on 11 protein targets indicates that the biphenyl motif is a preferred substructure for protein binding [7].

1.2
Historical Perspective

1.2.1
Early Considerations About Structure–Activity Relationships

In his interesting Edelstein award lecture, presented at the 224th American Chemical Society Meeting in Boston, MA, in August 2002 and entitled "To Bond or Not to Bond: Chemical Versus Physical Theories of Drug Action", John Parascandola [8] relates the early history of structure–activity relationships.

Regarding drug selectivity, he cites Earles, who states: "The fact that drugs may exert a selective action on specific organs of the body had long been recognized empirically and expressed vaguely in the traditional designation of certain remedies as cordials (acting on the heart), hepatics (acting on the liver), etc." [9].

One of the earliest to recognize structure–activity relationships was Robert Boyle in 1685, who tried to explain the specific effects of drugs in terms of mechanical philosophy by suggesting that since the different parts of the body have different textures, it is not implausible that when the corpuscles of a substance are carried by the body fluids throughout the organism, they may, according to their size, shape and motion, be more fit to be detained by one organ than another [10].

Later, at the turn of the 20th century, the German scientist Sigmund Fränkel argued that the selective action of drugs can only be understood by assuming that certain groups in the drug molecule enter into a chemical union with the cell substance of a particular tissue. Once fixed in the cell in this manner, the drug can exert its pharmacological action [11].

Despite this pioneering view, the understanding of the nature of chemical bonding and of cellular structure and function was still in its infancy at the beginning of the 20th century. Thus there was significant controversy over whether the physical or the chemical properties of a substance could best explain its pharmacological action and over the value of attempts to relate the physiological activity of a drug to its chemical structure. As an example, in 1903 Arthur Cushny, Professor of Materia Medica and Therapeutics at the University of Michigan, published a paper in the *Journal of the American Medical Association* entitled "The pharmacologic action of drugs: is it determined by chemical structure or by physical characters?" [12]. To a chemist today, such a question might seem odd. Finding convincing answers to it became possible only after the discovery of the existence and role of pharmacological receptors.

1.2.2
Early Considerations About the Concept of Receptors

The idea that drugs act upon receptors began with Langley in 1878 [13], who introduced the term "receptive substance" [14]. However, the word "receptor" was introduced later, by Paul Ehrlich [15, 16]. During the first half of the 20th century, several observations highlighted the critical features associated with the concept of receptors [17].

"Three striking characteristics of the actions of drugs indicate very strongly that they are concentrated by cells on small, specific areas known as receptors. These three characteristics are (i) the high dilution (often 10^{-9} M) at which solutions of many drugs retain their potency, (ii) the high chemical specificity of drugs, so discriminating that even D- and L-isomers of a substance can have different pharmacological actions, and (iii) the high biological specificity of drugs, e.g. adrenaline has a powerful effect on cardiac muscle, but very little on striatal muscle." [17].

1.2.3
Ehrlich's "Magic Bullet"

Selective interaction of a drug molecule with the corresponding receptor was not always accepted. One of the most brilliant demonstrations came from Paul Ehrlich's discovery of salvarsan, which gave rise to the concept of a chemotherapeutic "magic bullet" against specific infectious organisms. Beginning with dyes and later extending his studies to include arsenical compounds, Ehrlich modified the chemical structure of numerous molecules to produce effective drugs against trypanosome and later spirochete infections. They tested hundreds of

compounds before they came upon one, number 606, that Ehrlich thought was the chemotherapeutic agent he was searching for. Clinical tests confirmed the potential of the drug in treating syphilis and trypanosomiasis. The discovery was announced in 1910. Ehrlich named the drug salvarsan. The German physician, bacteriologist and chemist Paul Ehrlich shared the Nobel Prize in 1908 with Ilya Metchnikoff for their contributions to immunity.

1.2.4
Fischer's "Lock and Key"

Ehrlich's seminal discoveries reinforced the assertion made in 1894 by another brilliant German chemist, Emil Fischer. In a publication dealing with the effect of glucoside conformation on the interaction with enzymes, he wrote: "Um ein Bild zu gebrauchen, will ich sagen, dass Enzym und Glucosid wie Schloss und Schlüssel zu einander passen müssen, um eine chemische Wirkung auf einander ausüben zu können" (To illustrate, I would like to say that enzyme and glucoside must fit together like lock and key, in order to have a chemical effect on each other) [18]. The image of "lock and key" is still used today, even if it suggests a rigid structure of the receptor or enzyme protein. Probably another image, such as "hand in a glove", would be more accurate. Effectively, in addition to the steric complementarity, it would account for chirality and receptor flexibility.

1.3
Pharmacophores: the Viewpoint of a Medicinal Chemist

Even before the advent of computer-aided drug design, simple pharmacophores were described in the literature and considered as tools for the design of new drug molecules. Initial structure–activity relationship considerations were accessible in the 1940s thanks to the knowledge of the bond lengths and the van der Waals sizes which allowed the construction of simple two-dimensional model structures. With the availability of X-ray analysis and conformational chemistry, access to three-dimensional models became possible in the 1960s.

1.3.1
Two-dimensional Pharmacophores

1.3.1.1 Sulfonamides and PABA

The recognition of the quantitatively almost unmatched ability of *p*-aminobenzoic acid (PABA) to oppose the bacteriostatic efficiency of the sulfonamides led Woods and Fildes [19, 20] to formulate the fundamentals of the theory of metabolite antagonism (Fig. 1.1).

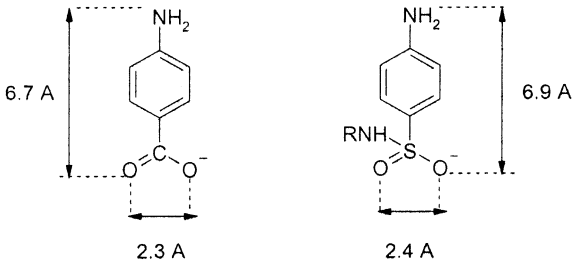

Fig. 1.1 PABA and *p*-aminobenzenesulfonamide show similar critical distances. The incorporation of the sulfonamide instead of PABA inhibits the biosynthesis of tetrahydrofolic acid.

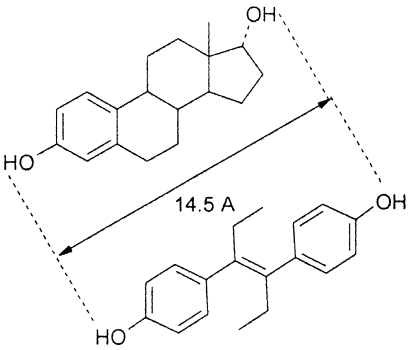

Fig. 1.2 Analogy between estradiol and *trans*-diethylstilbestrol.

1.3.1.2 **Estrogens**

Another early achievement (Fig. 1.2) was the synthesis and the pharmacological evaluation of *trans*-diethylstilbestrol as an estrogenic agent showing similarities with estradiol [21]. Here again the proposed model was two-dimensional [22], despite the fact that the non-planar conformation of estradiol was already known.

1.3.2 **An Early Three-dimensional Approach: the Three-point Contact Model**

When an asymmetric center is present in a compound, it is thought that the substituents on the chiral carbon atom make a three-point contact with the receptor. Such a fit insures a very specific molecular orientation which can only be obtained for one of the two isomers (Fig. 1.3). A three-point fit of this type was first suggested by Easson and Stedman [23], and the corresponding model proposed by Beckett [24] in the case of (*R*)-(–)-adrenaline [= (*R*)-(–)-epinephrine]. The more active natural (*R*)-(–)-adrenaline establishes contacts with its receptor through the three interactions shown in Fig. 1.3.

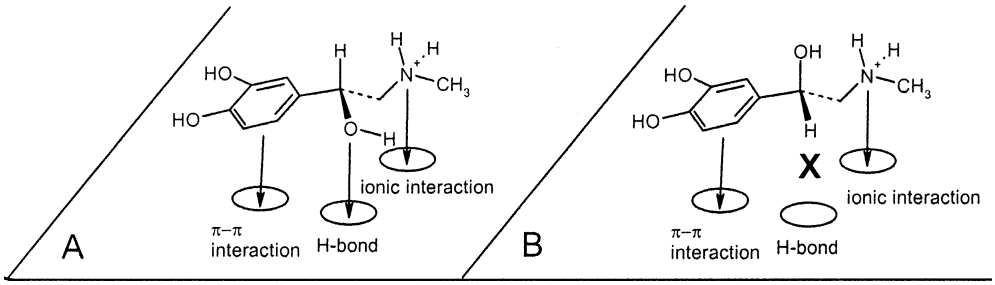

Fig. 1.3 Interaction capacities of the natural (*R*)-(–)-epinephrine and its (*S*)-(+)-antipode.

In simply assuming that the natural (*R*)-(–)-epinephrine establishes a three-point interaction with its receptor (A), the combination of the donor–acceptor interaction, the hydrogen bond and the ionic interaction will be able to generate energies of the order of 12–17 kcal mol^{-1}, which corresponds [25] to binding constants of 10^{-9}–10^{-12}. The less active isomer, (*S*)-(+)-epinephrine, may establish only a two-point contact (B). The loss of the hydrogen bond interaction equals $\sim$3 kcal mol^{-1}, hence this isomer should possess an $\sim$100-fold lesser affinity. Experience confirms this estimate. If we consider less abstract models, it becomes apparent that the less potent enantiomer also is able to develop three intermolecular bonds to the receptor, provided that it approaches the receptor in a different manner. However, the probability of this alternate binding mode to trigger the same biological response is close to zero.

1.3.2.1 Clonidine and Its Interaction with the α-Adrenergic Receptor

In the early 1970s, it was accepted that the hypotensive activity of clonidine was due to its direct interaction with the central norepinephrine receptor [26]. To trigger the α-adrenergic receptor, it was accepted that norepinephrine binds to its receptor by means of three bonds [27, 28]:

1. an ionic bond between the protonated amino function and an anion (carboxylate, phosphate) of the receptor active site;
2. a hydrogen bond between the secondary alcoholic hydroxyl and a, NH–CO function of the receptor;
3. a stacking (or charge transfer?) between the aromatic ring and an electron-deficient ring such as a protonated imidazole of a histidine residue.

In addition, it was known that the phenolic hydroxyls are not essential for α activity and that the cationic head should not be too bulky.

Pullmann et al. [29], in their model of the α-adrenergic receptor, found the following critical intramolecular distances: D=5.1–5.2 Å from N^+ to the center of the aromatic ring and H=1.2–1.4 Å for the elevation of the positive charge to the plane of the aromatic ring (Fig. 1.4).