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REVIEW ARTICLE

STRUCTURE BASED DRUG DESIGN: A REVIEW

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ABSTRACT

The field of structure-based drug design is a rapidly growing area in which much advancement has occurred in recent years. The outburst of genomic, proteomic, and structural information has provided hundreds of new targets and opportunities for future drug lead finding. This review condenses the process of structure-based drug design and comprises, primarily, the choice of a target, the evaluation of a structure of that target, the essential queries to consider in choosing a method for drug lead discovery, and evaluation of the drug leads.

Keywords: Drug discovery, drug target, leads complex, receptor, modelling

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Introduction

During the early 1980s, the ability to rationally design drugs using protein structures was an unrealized goal for many structural biologists. The first projects were underway in the mid-80s, and by the early 1990s the first success stories were published.[1,2,3] Today, even though there is still quite a bit of fine-tuning necessary to perfect the process, structure-based drug design is an integral part of most industrial drug discovery programs [4] and is the major subject of research for many academic laboratories.

The completion of the human genome project, the start of both the proteomics and structural genomics revolutions, and developments in information technology are fuelling an even greater opportunity for structure-based drug design to be part of the success story in the discovery of new drug leads. Excellent drug targets are identified at an increased pace using developments in bioinformatics. The genes for these targets can be cloned quickly, and the protein expressed and purified to homogeneity. Advances in high-throughput crystallography, such as automation at all stages, more intense synchrotron radiation, and new developments in phase determination, have shortened the timeline for determining structures. Structure determination using nuclear magnetic resonance (NMR) has also seen a number of advances in the past years, including magnet and probe improvements, automated assignment [5,6,7,] and new experimental methods to determine larger structures[8], Faster computers and the availability of relatively inexpensive clusters of computers have increased the speed at which drug leads can be identified and evaluated *in silico*.

Structure-based drug design is most powerful when it is a part of an entire drug lead discovery process. A review by J. Antel[9]states that the combination of combinatorial chemistry and structure-based design can lead to the parallel synthesis of focused compound libraries. It is also important to consider that structure-based drug design directs the discovery of a drug lead, which is not a drug product but, specifically, a compound with at least micromolar affinity for a target [11]. The time devoted to the structure-based drug design process, as outlined in this review, may represent only a fraction of the total time toward developing a marketable drug product. Many years of research may be necessary to convert a drug lead into a drug that will be both effective and tolerated by the human body. Additional

years of research and development will bring the drug through clinical trials to finally reach the market.

This review is intended to provide an overview of the process of structure-based drug design from the selection of a target to the generation and evaluation of lead compounds. An in-depth discussion or evaluation of the computational methods involved in drug discovery will not be provided here, since that subject has been covered in reviews elsewhere [12,13,14,15,16].

Overview of the Process

The process of structure-based drug design is an iterative one (Figure 1) and often proceeds through multiple cycles before an optimized lead goes into phase I clinical trials. The first cycle includes the cloning, purification and structure determination of the target protein or nucleic acid by one of three principal methods: X-ray crystallography, NMR, or homology modeling. Using computer algorithms, compounds or fragments of compounds from a database are positioned into a selected region of the structure. These compounds are scored and ranked based on their steric and electrostatic interactions with the target site, and the best compounds are tested with biochemical assays. In the second cycle, structure determination of the target in complex with a promising lead from the first cycle, one with at least micromolar inhibition *in vitro*, reveals sites on the compound that can be optimized to increase potency. Additional cycles include synthesis of the optimized lead, structure determination of the new target:lead complex, and further optimization of the lead compound. After several cycles of the drug design process, the optimized compounds usually show marked improvement in binding and, often, specificity for the target.

Choice of a Drug Target

The choice of a drug target is primarily made on a biological and biochemical basis. The ideal target macromolecule for structure-based drug design is one that is closely linked to human disease and binds a small molecule in order to carry out a function. The target molecule usually has a well-defined binding pocket. Other designed small molecules can compete, at a required level of potency, with the natural small molecule in order to modulate the function of the target. Many good drug targets are proteins; however, drug design against RNA targets with well-defined secondary structure, like the bacterial ribosome and portions of the HIV genome, has also been effective. Recent reviews highlight some of the RNA structure-based projects underway

[18,19,]In diseases caused by the malfunction of human proteins, small molecule drugs against G protein coupled receptors (GPCRs) represent at least 25% of the currently marketed drugs [20]. Small molecules that modulate the function of ion channels, proteases, kinases, and nuclear hormone receptors make up another 22% of the market.

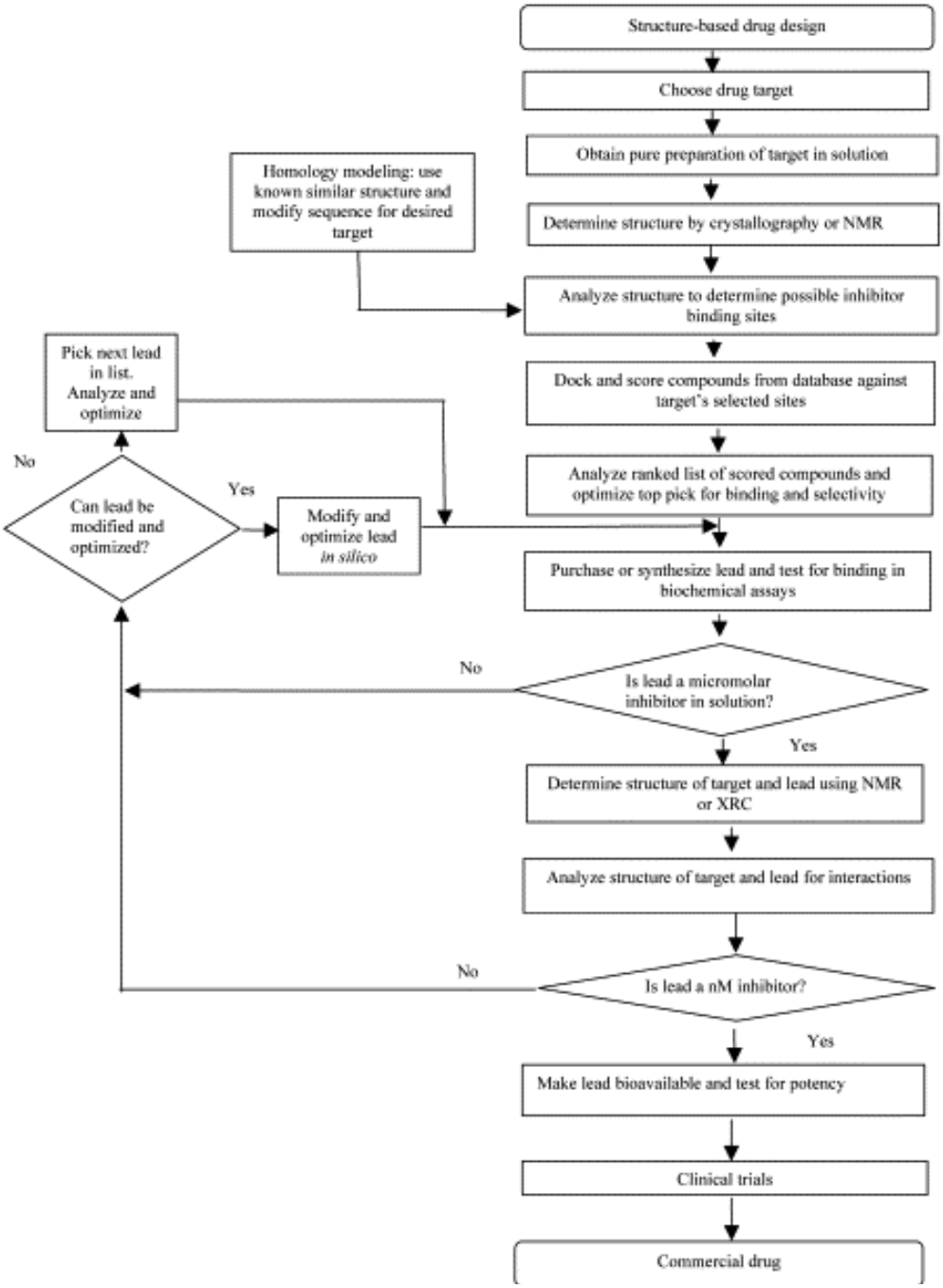

Figure 1

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The goal in developing drugs against the targets listed above is often to modulate the function of the human protein; the goal in developing drugs against pathogenic organisms is total inhibition, leading to the death of the pathogen. Antimicrobial drug targets should be essential, have a unique function in the pathogen, be present only in the pathogen, and be able to be inhibited by a small molecule. The target should be essential, in that it is part of a crucial cycle in the cell, and its elimination should lead to the pathogen's death. The target should be unique: no other pathway should be able to supplement the function of the target and overcome the presence of the inhibitor. If the macromolecule satisfies all outlined criteria to be a drug target but functions in healthy human cells as well as in a pathogen, specificity can often be engineered into the inhibitor by exploiting structural or biochemical differences between the pathogenic and human forms. Finally, the target molecule should be able to be inhibited by binding a small molecule. Enzymes are often excellent drug targets because compounds can be designed to fit within the active site pocket.

Cancer targets can be difficult because the targets are often somatic cell mutants of proteins that regulate essential cellular functions, resulting in the loss of a function. Of course, it is difficult for a small molecule to potentiate the recovery of a function. However, as

pointed out in a perspective by W. Kaelin[21]a loss of function in one molecule is often correlated with a gain of function in another. The disruption of oncogenic complexes is another difficult problem for anticancer drug design. For example, a chromosomal translocation in core binding factor β causes the formation of a novel chimeric protein that sequesters necessary transcription factor subunits[22]. Despite the difficulty of designing a small molecule to disrupt an unwanted protein association, the specific interface between the fusion protein and the transcription factor does provide a target that can be exploited. Finally, malignancy often alters the target from its normal behavior, leading to interest in the design of specificity for the malignant state.

Evaluating a Structure for Structure-Based Drug Design

Once a target has been identified, it is necessary to obtain accurate structural information. There are three primary methods for structure determination that are useful for drug design: X-ray crystallography, NMR, and homology modeling. The evaluation of structures from each method will be discussed.

Drug Design Methods

Once the structure and target site are identified, there are several paths to developing a good lead based on the structure of the target. These paths can be broadly classified as computer aided versus experimental. Computer-aided methods will be the main focus of this review. An example of an experimental method, by way of contrast, is high-throughput screening with combinatorial chemistry, in which thousands of compounds are tested for biochemical effects.

The computer-aided methods can be further classified into at least three categories: inspection, virtual screening, and de novo generation. In the first category, inspection, known molecules that bind the site, such as substrates or cofactors in the case of enzymes, or peptides in the case of protein:protein or protein:nucleic acid interactions, are modified to become inhibitors based on maximizing complementary interactions in the target site [1,3,]. In virtual screening, databases of available small molecules are docked into the region of interest *in silico* and scored based on predicted interactions with the site. Finally, for de novo generation small fragments of molecules, such as benzene rings, carbonyl groups, amino groups, etc., are positioned in the site, scored, and linked *in silico*. The final compounds, created *in silico* from the linked fragments, then must be synthesized in the laboratory. There is some overlap between the

virtual screening and de novo generation classifications. Some programs, for example, LUDI, which is usually used to dock fragments of compounds, are also capable of docking and scoring entire compounds. The programs are classified in Table 1 according to their primary use.

There are many excellent drug design software methods available capable of either virtual screening or de novo generation. This review will focus on a few of the major points necessary to decide on a particular route for lead generation. Extensive reviews of the software are available [11,12,13,14,] and are highly recommended for further reading. Questions that are essential in deciding on a method for lead generation are as follows: (1) are molecules available which can be modified to be inhibitors, (2) is there a means for synthesizing novel molecules, and (3) what is the degree of accuracy required at a particular stage of the design process versus the time needed for the calculation? Factors such as the inclusion of protein or ligand flexibility and the effects of solvent increase the time needed for the calculation but also increase the predictive value. Each of these questions will be discussed with reference to available drug design algorithms.

Modifying an Initial Compound

Substrates and cofactors for many proteins have been modified to become excellent inhibitors [1,3](Figure2 for an example). Initially, the crystal structure is solved in the presence of a substrate, cofactor, or drug lead. Then, modifications to direct the small molecule toward being a potent inhibitor are designed *in silico* based on the interactions of the molecule with the target site. The newly designed compounds are then scored for binding using evaluative scoring algorithms available in virtual screening methods.

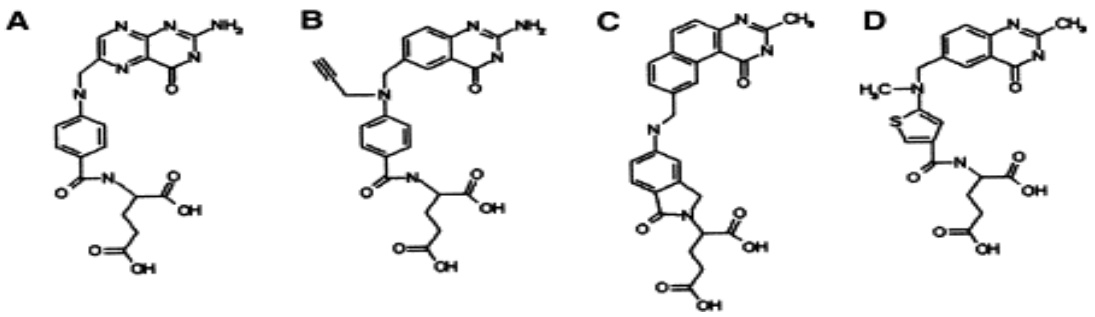

Figure 2

Inhibitors for ThymidylateSynthase were Designed Based on Modifications of the Cofactor 5,10-Methylene Tetrahydrofolate

Drug Lead Evaluation

Once a small molecule has been identified as potentially binding to the target molecule, it must be evaluated before proceeding to further stages. It is important to consider that the ranking assigned by the scoring function is not always indicative of a true binding constant, since the model of the target:ligand interaction is inherently an approximation. Both the solvent effect and the effects of target and ligand flexibility are usually imprecisely described. Usually, several molecules which scored well during the docking run are evaluated in further tests since even the top scoring molecule could fail in vitro assays. Leads are first evaluated visually with computer graphics and can often be optimized at this step for increased affinity. Leads are also evaluated for their likelihood to be orally bioavailable using the "Rule of 5".which states that good leads generally have less than five hydrogen bond donors and less than ten hydrogen bond acceptors, a molecular weight less than 500, and a calculated log of the partition coefficient (clogP) less than 5. Rigidifying the lead can also impart a lower binding constant by decreasing the conformational entropy in the unbound state to approach the presumably very low conformational entropy in the bound state. Veber and colleagues state that the number of rotatable bonds should be less than ten in order to increase the potential for oral bioavailability. Other factors, such as chemical and metabolic stability and the ease of synthesis, can also factor into the decision to proceed with a particular candidate lead. Finally, leads are brought into the wet lab for biochemical evaluation.

Promising leads re-enter the structural determination process to find the exact binding mode and to evaluate any further optimization that becomes evident. A few examples of designed leads have shown significant differences between predicted and actual binding modes but in many cases the docked and experimental conformations are within 2 Å rmsd16

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