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NMR screening and studies of target – ligand interactions

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Nuclear magnetic resonance (NMR) spectroscopy is one of the leading biophysical methods used in the search for and design of physiologically active compounds considered as potential drugs. The review concerns modern NMR techniques used to study the binding of low-molecular-mass compounds to biomacromolecular targets. The most promising methods of NMR screening and strategies for rational lead design are discussed. They were used to design drugs that have been approved for the use in medical practice or are in the final stages of clinical trials. Examples are given of the application of the fragment-based drug design and NMR screening techniques to the design of novel drugs.
The bibliography includes 252 references.

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1. Introduction

In the early 20th century, rapid progress in natural sciences and the development of novel methods of synthesis and structure determination of organic compounds led to a situation where the search for novel drugs got a strong chemical basis. Since then drug design has involved the synthesis of novel structural analogues of metabolites, endogenous compounds, hormones, *etc.* rather than simple application of the empirical method known since ancient times. A hundred years later the science of novel drug design is based on biology rather than chemistry. To date, key biological targets have been established, methods for structure determination of biomolecules (X-ray crystallography, nuclear magnetic resonance, cryoelectron microscopy) developed, genetic engineering methods for production, isolation and purification of designed biomacromolecules, as well as methods of investigation of interactions between the targets and potential drugs. Target-oriented methods of search for novel lead compounds have become a basis for modern drug design strategies.[1, 2]

Key factors simulating research activity in this field include expansion of the list of diseases, drug resistance and identification of new side effects of the known drugs. The development of a novel drug is a multistep process that requires huge, constantly growing time demand and cost (Fig. 1).† Leading pharmaceutical companies estimate that the development of an innovative drug may take 10 to 15 years and >1 billion US dollars.[3] The overall cost is mainly spent for long-term and expensive phases of clinical trials of drugs, where cost reduction is impossible since the requirements for the safety and efficacy of the drugs being designed are continuously toughened.[4, 5] Up to 25% of time and cost are spent for the initial steps of hit discovery and hit to lead optimization. These expenses can be reduced by using modern

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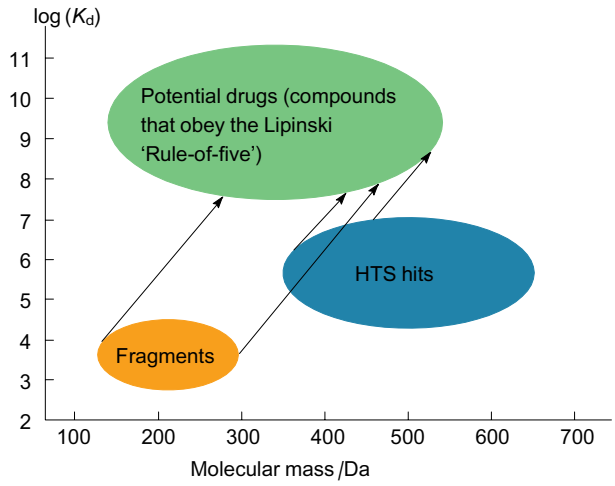

Figure 31. Assessment of approaches to the design of hit compounds based on total screening and FBLD in the coordinates K_d is molecular mass of the complex with the target.
The characteristic regions for the hits that obey the Lipinski rules, HTS hits, and molecular fragments binding to targets are given in colour. Arrow show directions of potency optimization of compounds through an increase in affinity and molecular mass. Based on the illustration from Ref. 252.

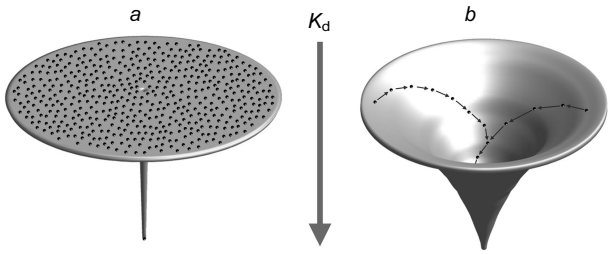

Figure 32. Schematic representation of the total screening (*a*) and fragment-based hit design (*b*) methods.
Surfaces denote the diversity of chemical structures. The depth of each point is proportional to the binding constant of corresponding compound to the target protein. Total screening aimed at finding one or more hits requires experimental studies of thousands of compounds (conditionally represented by dots). The fragment-based method combined with rational structure design strategies (including NMR screening methods) requires the synthesis and experimental studies of much smaller number of compounds. The concept of this Figure is based on the illustration from Ref. 217.

active compounds. It also covers the principles of design of fragment libraries as well as methods for selection of active fragments and subsequent lead design.

Mention should be made of differences between two modern approaches to the search and design of drugs, *viz.*, high-throughput total screening and fragment-based strategy. Figure 31 illustrates them as applied to lead design.

The plot shows regions typical of potential drugs that obey the Lipinski rules, HTS hits and structural fragments capable of binding to targets. Usually, such fragments have a mass of 150–300 Da and relatively low affinity. The affinity of the HTS hits is much higher; however, their molecular masses are also higher. To become promising lead compounds, both structural fragments and HTS hits should be modified. Structure modification of both classes of compounds is aimed at increasing their affinity to target proteins. It is accompanied by complication of the molecular structure and, most often, by an increase in the molecular mass. Thus, the optimization of compounds can be represented as the motion along the direction specified by the arrows in Fig. 31. The probability to reach the region of potential drugs is much higher for the modified fragments rather than HTS leads.

These methods of rational lead design based on NMR spectroscopy data allow one to modify molecular fragments by optimizing their properties in step-by-step manner. This feature differs the strategy in hand from the search for high-affinity compounds using HTS (Fig. 32).

High-throughput screening (see Fig. 32 *a*) requires testing thousands of compounds until a hit (or a few hits) with high affinity for the target protein are selected. In this case, the probability to reach a narrow high-affinity region is very small. The fragment-based method (Fig. 32 *b*) allows one to begin the design of a potential lead compound using one or a few fragments with relatively low affinity for the target. Subsequent fragment modification and linking will lead to an increase in the affinity of the optimized compound. By gradually varying the structure of the ligand it is possible to move along the gradient descent direction towards minimum values of the dissociation constant of the ligand–target complex. The surfaces shown in Fig. 32 denote the diversity of chemical structures while the shape of the surface is determined by the binding constants of individual compounds. Thus, the fragment-based method and NMR screening are rational approaches to the design of biologically active compounds. Undoubtedly, these methods will play an increasingly important role in the design of novel drugs.

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