4

NMR in fragment-based drug discovery

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AN INTRODUCTION TO FRAGMENT-BASED LEAD DISCOVERY

For decades, molecular starting points for drug discovery have been found by screening large numbers of natural and synthetic compounds for biological activity in phenotypic and biochemical assays. Then, beginning in the mid to late 1990s, several pharmaceutical groups developed new approaches [such as structure/activity relationship by nuclear magnetic resonance (SAR by NMR),[1] the SHAPES strategy,[2] and needle screening[3]] in which simple, low-molecular-weight compounds were screened for binding to the target of interest, and these relatively weak binding molecules were then used to systematically construct larger, more potent, drug leads. Such small screening molecules are now commonly called fragments and the related processes collectively called fragment-based lead discovery (FBLD).

NMR was the first experimental method used to screen fragments, and although a variety of other techniques (including x-ray crystallography, surface plasmon resonance, high concentration bioassays, and mass spectroscopy) have also been applied, NMR is still the most widely used. This chapter reviews the use of NMR for FBLD, beginning with an explanation of the principles behind fragment screening. NMR screening methods are then described, followed by a series of examples that illustrate the process through which fragment screening hits are converted into leads.

Defining fragments

To explain the principles of FBLD, it is first necessary to define what molecules are considered to be fragments. Molecules are often classified using their physicochemical properties, such as molecular weight (MW), number of hydrogen bond donors and acceptors (HBD and HBA), calculated log*P* (Clog*P*), and number of rotatable bonds. A popular rule of thumb for classifying molecules as "druglike" is the "rule-of-five" first described in a well-known article from Pfizer.[4] This study reported that a compound is more likely to exhibit poor oral absorption and cell permeability when its properties exceed limits (Table 4.1) defined by four simple rules (dubbed the "rule-of-five" because the values are multiples of five). Approximately 90% of the approximately 2,000 Phase II clinical candidates analyzed satisfied at least three of the four rules.

Because lead optimization by medicinal chemists typically results in molecules that are larger and more lipophilic than the starting lead, it has been proposed[5,6] that the compounds screened should be "leadlike" rather than "druglike," with properties well within the rule-of-five limits to allow room for subsequent improvements in potency, selectivity, and absorption/distribution/metabolism/excretion (ADME) properties (Table 4.1).

Fragment-based screening evolved in parallel with the practice of classifying compounds as drug- or leadlike according to their physicochemical properties, so the much smaller molecules used for FBLD were defined using the same parameters. The term *fragment* originates from the practice of computationally dividing known biologically active molecules into their basic building blocks[7,8] and then using those components for screening. Although there is a general consensus among practitioners of FBLD regarding which properties should be used to define fragments, there is some disagreement about the preferred values. The group at Astex Therapeutics has reported that the hits obtained from crystallographic screening of their MW = 100- to 250-Da fragment library seem to conform to a "rule-of-three" (Table 4.1).[9] The rule-of-three has been adopted by many groups and commercial vendors for designing fragment libraries, particularly those used for crystallography-based screening. Some groups, particularly those using NMR-based screening methods, find that rule-of-three compliant libraries are too limited and produce hits that are so small and simple that they are difficult to optimize synthetically, because of very weak (e.g., multimillimolar) affinities, a lack of synthetically accessible functional groups, and the need for structural information to guide synthesis. To address this, they employ higher limits for molecular weight, HBA, and rotatable bonds to create libraries of "reduced complexity" leads or scaffolds (Table 4.1).[10–12] A minimum MW cutoff of ~150 Da is also often used to avoid the possibility of binding in multiple orientations, a problem that occurs more frequently for the smallest and simplest fragments.[13–15] In addition to the properties listed in