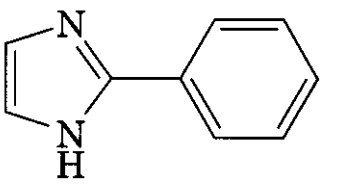
individual compounds within the mixture. However, signals from the protein are always present, which can pose a problem in interpreting spectra. An isotope-edited version of the diffusion experiment has been designed to avoid this problem, although labeled protein is required (168). Generally, there is no requirement for labeling of the protein target or for the protein resonances to be assigned and thus, in theory, there is no size limit on the proteins that can be screened by use of this method, although no information is obtained on the location of ligand binding. However, if the protein is large, then the transverse relaxation time may be too short to observe the bound ligands in the diffusion-edited spectrum (169). Only one sample, containing protein and ligands, is used to obtain both reference and screening data and therefore differences between the sample and reference spectra caused by addition of the ligands (pH, salt concentration, etc.) are avoided.

Diffusion-filtered NMR screening requires that there is a significant difference in observed translational diffusion between the free and bound states. The ligands are in fast exchange on the diffusion timescale and as a consequence the observed diffusion coefficient for binding ligands is an average between the free and bound diffusion values. Free ligands diffuse at a much faster rate than those in the bound state and thus only a small amount of free ligand has a considerable effect on the observed average diffusion coefficient. This effect may be significant enough to reduce the difference between binding and nonbinding ligands, making it more difficult to interpret results (169). It has also been demonstrated that chemical exchange and NOE can affect the interpretation of diffusion experiments and that these factors need to be taken into consideration (170, 171).

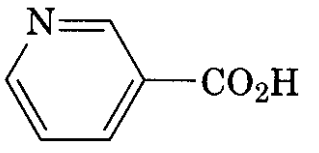
Shapiro and coworkers developed a methodology based on diffusion filtering, named "affinity NMR," that they have used to screen for binding (172–175). Diffusion-edited NMR experiments were able to identify two known binding tetrapeptide ligands of vancomycin from a mixture of 10 peptides (176). Hajduk et al. demonstrated the application of diffusion-editing experiments by differentiating ligands of stromelysin from a mixture containing nonbinding compounds (177).

4.1.4 Relaxation. Like diffusion, the transverse relaxation time (T_2) of molecules is also dependent on molecular size. Large molecules, such as proteins, have a short T_2 and hence exhibit broad NMR signals, whereas small molecules have a longer T_2 and hence narrower line widths. Therefore, if a small molecule ligand binds to a protein, its T_2 value will decrease and a line-broadening effect of bound ligand signals can be observed. Alternatively, a relaxation filter can be used to remove signals from molecules with a short T_2 value. Subtraction from a reference spectrum will result in a spectrum containing only those ligands that bind to the protein.

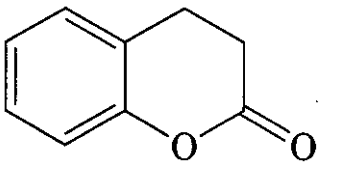
The ability to identify binding ligands using relaxation filters has been demonstrated using FKBP. A mixture of nine compounds consisting of one known ligand of FKBP, 2-phenylimidazole (**34**), and eight nonbinding compounds (e.g., 35–37 were screened and only signals from (**34**) were observed (177).



(34)



(35)



(36)

4.1.5 NOE. NOE experiments can also be used to identify ligands that bind to protein targets (178–180).Small molecules have a fast