Privileged Molecules for Protein Binding Identified from NMR-Based Screening

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A statistical analysis of NMR-derived binding data on 11 protein targets was performed to identify molecular motifs that are preferred for protein binding. The analysis indicates that compounds which contain a biphenyl substructure preferentially bind to a wide range of proteins and that high levels of specificity (>250-fold) can be achieved even for these small molecules. These results suggest that high-throughput screening libraries that are enriched with biphenyl-containing compounds can be expected to have increased chances of yielding high-affinity ligands for proteins, and they suggest that the biphenyl can be utilized as a template for the discovery and design of therapeutics with high affinity and specificity for a broad range of protein targets.

Introduction

The concept that particular molecules have a high propensity for binding to proteins has been an area of interest for many years. The identification of such privileged molecules could be useful for enriching high-throughput screening libraries with compounds that contain these preferred substructures. This could be accomplished by purchasing select compounds or by synthesizing directed libraries that contain these moieties. Several approaches have been employed to identify privileged substructures from the database of known drugs using pharmacophore modeling techniques and a variety of fragmentation algorithms.[1,2] Interestingly, one study identified a set of 32 molecular substructures that described ~50% of all known drugs.[3] More recently, biologically privileged motifs have been identified for specific therapeutic classes using a retrosynthetic analysis.[4]

Here we describe the identification of small molecules that preferentially bind to proteins from a statistical analysis of NMR-derived binding data for a diverse set of small molecules tested against a variety of protein targets. NMR is uniquely suited to identify ligands for proteins[5−10] and has been used to discover novel leads for a number of protein targets using a library of 1000−10 000 diverse small molecules with an average molecular weight of 200 Da. [5,11−14] Unlike larger, more flexible molecules, these small compounds have a limited number of functional groups and rotatable bonds which simplify the interpretation of binding data. Although low molecular weight compounds would be expected to bind only weakly to proteins, NMR is a robust and reliable technique for measuring weakly bound ligands.

Results and Discussion

Generation of Fragments. As a first step, common classes of fragments were selected which can be used to represent the compound library. This was accomplished using a modification of the RECAP algorithm[4] which cleaves the chemical bonds of the molecules in the database until only fragments remain. A total of 104 fragments were identified to represent the compounds in the library. More than 99% of the 10 080 compounds in the NMR library contained at least one of these substructures, indicating that the set of 104 fragments is a good representation of the library. A table was prepared that contained each of the 10 080 compounds in the library associated with the substructures that were contained within the molecule along with a binary representation (active/inactive) of the binding data for each of the proteins.

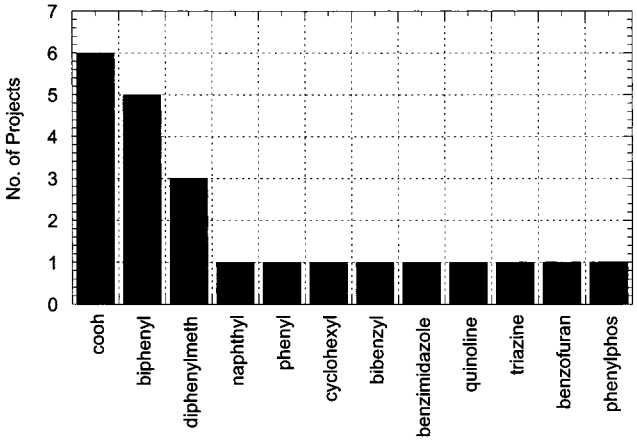

Figure 1. Number of projects (out of 11) for which a particular substructure was enriched in the active compounds (see text for details).

Privileged Substructures. For this analysis, positive and negative binding from NMR-based screening was analyzed for 11 proteins (see Experimental Section). A statistical procedure was applied to the data to examine the increased occurrence of particular substructures in the active compounds (see Experimental Section). A total of 12 fragments were identified whose representation was significantly enriched for at least one of the 11 proteins (Figure 1 and Table 1). While the majority of these fragments are statistically preferred for binding to only a single protein target, the carboxylic acid and biphenyl substructures were found to preferentially bind to nearly 50% of the proteins tested. For the carboxylic acid group, the enrichment factors over chance, Δ, ranged from 6 to 45 for six of the protein

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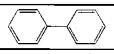
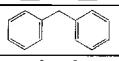
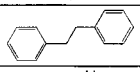
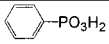
Table 1. Privileged Substructures Identified in an Analysis of NMR-Derived Binding Data

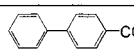
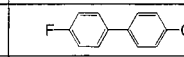
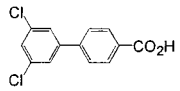
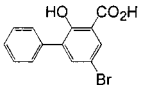
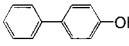
Name	Structure	#Protein Targets[a]	Δ[b]	β[c]	%Library[d]	%Drugs[e]	%ACD[f]
cooh	CO_2H	6 (55%)	6.1-44.7	0.59-2.03	12.6	19.4	8.1
biphenyl		5 (45%)	5.5-99.7	2.15-2.40	1.9	4.3	2.7
diphenylmethane		3 (27%)	12.1-13.8	1.00-1.31	5.2	8.6	6.4
naphthyl		1 (9%)	6.4	1.01	1.7	3.3	3.3
phenyl		1 (8%)	3.9	0.75	68.7	73.3	73.2
cyclohexyl		1 (9%)	3.4	1.51	4.5	12.2	6.3
bibenzyl		1 (9%)	6.5	0.73	3.0	5.9	6.0
benzimidazole		1 (9%)	17.5	1.61	1.0	0.8	0.3
quinoline		1 (9%)	34.3	2.13	0.9	4.2	1.5
triazine		1 (9%)	22.5	2.09	0.8	0.2	0.4
benzofuran		1 (9%)	22.5	0.83	0.5	0.8	0.3
phenyl-phosphonate		1 (9%)	98.8	3.52	0.2	0.03	0.04

[a] The number of projects (of 11) for which the substructure was represented significantly greater than chance in the active compounds. [b] Increase over chance of the substructure's representation in the active compounds. The statistical confidence limits for all entries is $p < 0.002$. Where the substructure was preferred against multiple targets, the range for Δ against these targets is given. [c] Regression coefficient from the logistic regression model. Where the substructure was preferred against multiple targets, the range for β against these targets is given. [d] Percentage of compounds (of 10 080) used in the analysis that contain this substructure. [e] Percentage of 154 000 compounds from the World Drug Index (Derwent) and Maccs Drug Data Report (MDL) that contain this substructure. [f] Percentage of 177 000 compounds from Available Chemicals Directory (MDL) that contain this substructure.

targets. This indicates that compounds containing a carboxylic acid were 6 to 45 times more likely to bind than compounds that lacked this group. This is not surprising since most of the protein targets that bound preferentially to compounds with carboxylic acids have DNA, RNA, or phosphorylated peptides as their natural ligands, and a chemical shift analysis indicated that these carboxylate-containing compounds were indeed binding at the natural ligand binding site. Thus, in these cases, the negatively charged carboxylic acid is likely to bind to the same site as the natural ligands and form similar electrostatic interactions. In contrast, the strong preference for protein binding of the biphenyl was unexpected and not as easily explained. Compounds that contained a biphenyl substructure were 5 to 100 times more likely to bind to 5 of the 11 proteins tested (Table 1). These proteins were very different and include enzymes, DNA-binding proteins, and proteins that interact with other proteins. As in the case with the carboxylic acids, it is interesting to note that a chemical shift analysis indicated that the biphenyls bound at or near the natural ligand sites on these proteins. The biphenyl moiety was present in 1.9% of the NMR compound collection, which is similar to the occurrence of naphthyl (1.7%), bibenzyl (3.0%), cyclohexyl (4.5%), and diphenylmethane (5.2%) substructures (Table 1). Thus, the strong preference for biphenyls as ligands for proteins is not an artifact of being over-represented in the library used to generate the binding data.

Specificity of Biphenyl Binding. Although the biphenyl moiety was found to preferentially bind to nearly half of the proteins tested, not all biphenyls bind to the same protein. In fact, specific and distinct

Table 2. Dissociation Constants (in mM) of Biphenyl-Containing Compounds for a Number of Protein Targets

No.	Structure	E2-DBD	Stromelysin	VEGF
1		2.5	>5	>5
2		1.5	>5	-[a]
3		0.06	-	>5
4		2.5	-	0.5
5		>5	0.3	>5
6		>5	0.02	>5

[a] Not determined.

structure−activity relationships were observed for the proteins to which they bind. For example, as shown in Table 2, biphenyls **1**−**3** bind to the DNA-binding domain of the HPV-E2 protein,[12] but bind 2- to 80-fold more weakly to either the matrix metalloproteinase stromelysin[11] or the receptor binding domain of the vascular endothelial growth factor (VEGF).[15] Even though VEGF and HPV-E2 both bind to biphenyl acids, specificity differences of more than 100-fold can be achieved by appropriate substitutions on the biphenyl (e.g., compare **3** with **4**). Biphenyls in which the carboxylic acid group is replaced by a hydroxyl group (e.g., **5**) preferentially bind to stromelysin compared to E2 or VEGF. This specificity is even more pronounced when a cyano group

(**6**) is added para to the biphenyl linkage, which results in 250-fold more selectivity for binding to stromelysin.

Rationale for Biphenyls as Privileged Ligands for Proteins. It has long been recognized that aromatic moieties are major players in molecular recognition. For example, the protein binding of drugs that contain aromatic substituents is dominated by interactions with aromatic and hydrophobic residues.[16,17] In addition, aromatics have been shown to form favorable interactions with polar substituents, such as amides[18] or hydroxyl groups,[19] and even positively charged moieties.[20,21] This most likely accounts for the fact that although only 58 of the 104 representative fragments contained an aromatic ring, 11 of the 12 privileged fragments contain this group (Table 1). It is interesting to note that phenyl itself was statistically preferred for only a single project, as the majority of other fragments contain multiple rings. This can be explained by the fact that some minimum number of interactions may be required to impart measurable binding energy, and compounds which contain only a single aromatic ring do not have a sufficient number of favorable contacts with the protein. However, the number of available interactions cannot explain the selection of biphenyls over naphthyl, diphenylmethane, or bibenzyl substructures, all of which are capable of approximately the same degree of aromatic interactions. One possibility for the preference for biphenyls is the degree of flexibility about the aromatic linkage. The naphthyl group is completely rigid, which severely limits the number and type of pockets it can occupy, while the greater flexibility of the bibenzyl and diphenylmethane substructures may result in entropic penalties upon binding. An alternative explanation may be that the biphenyl motif is simply of a size and shape that can conform to a wide variety of pockets that exist on protein surfaces.

Conclusions

In summary, a statistical analysis of NMR-derived binding data on 11 protein targets indicates that the biphenyl motif is a preferred substructure for protein binding. The data also suggest that although the biphenyl moiety imparts affinity for a wide range of proteins, high levels of specificity (>250-fold) can be achieved even for these small molecules. Thus, high-throughput screening libraries that are enriched with biphenyl-containing compounds would be expected to have increased chances of yielding high-affinity ligands for proteins. This enrichment can be achieved through the purchase of select compounds from commercial or private sources, or through the construction of biphenyl libraries using standard combinatorial chemistry or parallel synthetic approaches.[22] It is significant to note that the biphenyl substructure is found in 4.3% of all known drugs, and is used in a number of therapeutic areas (e.g., diflunisal[23] and valsartan[23]). All of these data indicate that the biphenyl can be successfully utilized as a template for the discovery and design of therapeutics with high affinity and specificity for a broad range of protein targets.

Experimental Section

Library Composition. The library was composed of 10 080 organic molecules with an average molecular weight of 200 Da. The compounds in the library were obtained from both internal and external sources and were picked to maximize the diversity of the molecules. The diversity of the final library was estimated based on Tanamoto coefficients as implemented in the Daylight Fingerprint Toolkit (see www.daylight.com for references and documentation). This analysis indicated that 80% of the compounds in the library are less than 80% similar to any other compound, and that any one compound in the database is on average 10% similar to every other compound in the database. A Ward's clustering analysis (Barnard Chemical Information, Ltd., see http://www.bci1.demon.co.uk/agglom.html for references and documentation) yielded an average cluster size of 1.5.

NMR Binding Data. NMR samples (and corresponding buffer conditions and sample temperatures) were obtained for the following proteins: (1) the DNA-binding domain of the human papillomavirus E2 protein (E2-DBD, 20 mM PO_4, 10 mM DTT, pH 6.5, $T = 37$ °C),[12] (2) the matrix metalloproteinase stromelysin (20 mM Tris, 20 mM $CaCl_2$, pH 7.0, $T = 30$ °C),[11] (3) the receptor binding domain of the vascular endothelial growth factor (VEGF, $T = 45$ °C),[15] (4) the FK-506 binding protein (50 mM PO_4, 100 mM NaCl, pH 6.5, $T = 30$ °C),[5] (5) the Src homology-2 domain of $p56^{lck}$ (50 mM Tris, 25 mM NaCl, 5 mM DTT, pH 7.2, $T = 30$ °C),[14] (6) the methyl transferase ErmAM (50 mM PO_4, 100 mM NaCl, 10 mM DTT, pH 6.5, $T = 30$ °C),[13] (7) the DNA-binding domain of NFATc (100 mM $NaPO_4$, pH 6.5, 50 mM KCl, 5 mM DTT, $T = 30$ °C),[24] (8) the peptidyl prolyl *cis*−*trans* isomerase Pin-1 (50 mM BisTris, 10 mM DTT, pH 6.5, $T = 30$°C),[25] (9) the anti-apoptotic protein Bcl-xL (10 mM PO_4, pH 6.5, $T = 37$ °C),[26] (10) the anti-apoptotic protein Bcl-2 (25 mM Tris, pH 7.5, $T = 37$ °C),[27] and (11) the phosphotyrosyl phosphatase PTP-1B (50 mM BisTris, 150 mM NaCl, 20 mM DTT, pH 6.5, $T = 30$ °C).[28] NMR samples were composed of uniformly ^{15}N-labeled protein at 0.3−0.5 mM in a H_2O/D_2O (9:1) solution. Bruker sample changers were used on Bruker AMX-, DMX-, and DRX-500 MHz spectrometers.

Ligand binding was detected by acquiring sensitivity-enhanced $^{15}N/^1H$−HSQC spectra[29] on 500 μL of protein in the presence and absence of added compound(s). Compounds were added as solutions in perdeuterated DMSO and were initially tested at 1.0 mM each in mixtures of 10. The mixtures of compounds were initially assigned a score ranging from 0 (no observed changes in the spectrum upon the addition of compounds) to 5 (significant changes in the spectrum). Individual compounds in mixtures that were assigned a score of 0 or 1 were designated "inactive." Individual compounds in mixtures that were assigned a score of 3 or higher were tested individually and assigned an individual score ranging from 0 to 5. Those compounds that were assigned a score of 0 or 1 were designated "inactive." Those compounds that were assigned a score of 3 or higher were designated "active." Mixtures or individual compounds that were assigned a score of 2 were designated as "ambiguous" and were not used in the analysis. Dissociation constants were obtained for selected compounds by monitoring the chemical shift changes as a function of ligand concentration. Data were fit using a single binding site model. A least squares grid search was performed by varying the values of K_D and the chemical shift of the fully saturated protein.

Fragment Generation. Fragment generation was accomplished using a modification of the RECAP procedure[4] in which the bonds of the molecules in the database were recursively cleaved until only the desired types of fragments remained. Lewell and co-workers used this procedure to find substructures commonly present in drug-like molecules, but the technique is useful for any database of organic compounds. In addition to the bond cleavage rules employed in RECAP, carbon−halogen bonds and nonring bonds between aromatic carbon atoms and aliphatic carbon, nitrogen, oxygen, and sulfur were also broken. The resulting fragments are typically simple one- or two-ring systems, either unsubstituted or containing one or two small substituents. A total of 104 fragments, including the 100 most populated fragment structures from the RECAP procedure plus four additional frag-

ments not generated through the bond cleavage rules (diphenylmethane, bibenzyl, carboxylic acid, and amine), were used in the analysis of the data. A complete list of the fragments used in the analysis is included in the Supporting Information.

Statistical Analysis. The NMR binding data was initially analyzed by constructing 104 tables (one for each of the representative substructures) for each of the 11 protein targets of the form below:

	no. active	no. inactive
substructure present	$n1$	$n2$
substructure not present	$n3$	$n4$

The hit ratio for the compounds containing the particular substructure ($n1/(n1 + n2)$) was then compared to the hit ratio for compounds lacking the substructure ($n3/(n3 + n4)$) using the ratio

$$\Delta = \frac{n1/(n1 + n2)}{n3/(n3 + n4)}$$

A value for Δ in excess of 1.0 indicates that a higher percentage of the active compounds contained that particular substructure when compared to those compounds that did not contain the substructure. Any deviation from 1.0 was tested for statistical significance using Fisher's exact test.[30]

A significant test result for any 2 × 2 table indicates that the substructure in question is more prevalent in active compounds than could be expected by chance alone. This is, however, no guarantee that the substructure by itself contributes to the activity of the compound; it may simply be that the substructure occurs together with (i.e., acts as a "proxy" for) another truly active substructure. For example, many of the targets which yielded biphenyls as significant substructures (p-value < 0.01) in the 2 × 2 table analysis also yielded benzene as significant. However, in many of these cases, the benzene substructure serves only as a proxy for the biphenyl (e.g., compounds that contained a biphenyl also contained a benzene moiety). To remove these proxy substructures, those fragments that gave p-values less than 0.01 were included as categories in a stepwise logistic regression model.[30] For each target, a sequence of binary variables Y_i (i = 1 to 10 080, for each of the tested compounds) was constructed where $Y_i = 1$ if compound i produced a hit and $Y_i = 0$ otherwise. A covariate vector $\mathbf{x}_j$ ($j = 1, ..., N$) was also constructed for each compound i, where $x_{ij} = 1$ if substructure j was present in compound i and −1 otherwise. Here, N is the number of substructures with p-values below 0.01 in Fisher's exact test.

Assuming that the compounds hit independently of each other and that each Y_i follows a binomial distribution, then the probability that compound i will hit is given by

$$P(Y_i = 1) = \frac{\exp(\alpha + \sum_j \beta_j x_{ij})}{1 + \exp(\alpha + \sum_j \alpha + \beta_j x_{ij})}$$

Here, $(\beta_1 ... \beta_{104})$ is the vector of unknown regression coefficients for the substructures, and α is a constant which gives the baseline hit rate against the target. A large and positive value of β_j indicates that the presence of substructure j increases the chance that a compound will hit, while negative values decrease the chance. The regression coefficients were initially estimated for the complete set of substructures that yielded p-values less than 0.01 in the 2 × 2 tables. Next, the least significant predictor (substructure) was eliminated from the model and the data refit. This was repeated until all remaining regression coefficients β_j were significant at confidence levels of 0.01%. The end result is a model in which all remaining substructures are significant at confidence levels of 0.01%.

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Supporting Information Available: Set of 104 fragments used in the analysis. This material is available free of charge via the Internet at http://www.pubs.acs.org.

References

(1) Klopman, G. Articificial Intelligence Approach to Structure−Activity Studies. Computer Automated Structure Evaluation of Biological Activity of Organic Molecules. *J. Am. Chem. Soc.* **1984**, *106*, 7315−7321.
(2) Leach, A. R. *Molecular Modelling: Principles and Applications*; Addison-Wesley Longman, Ltd.: Reading, MA, 1996.
(3) Bemis, G. W.; Murcko, M. A. The Properties of Known Drugs. 1. Molecular Frameworks. *J. Med. Chem.* **1996**, *39*, 2887−2893.
(4) Lewell, X. Q.; Judd, D. B.; Watson, S. P.; Hann, M. M. RECAP − Retrosynthetic Combinatorial Analysis Procedure: A Powerful New Technique for Identifying Privileged Molecular Fragments with Useful Applications in Combinatorial Chemistry. *J. Chem. Inf. Comput. Sci.* **1998**, *38*, 511−522.
(5) Shuker, S. B.; Hajduk, P. J.; Meadows, R. P.; Fesik, S. W. Discovering High-Affinity Ligands for Proteins: SAR by NMR. *Science* **1996**, *274*, 1531−1534.
(6) Lin, M.; Shapiro, M. J.; Wareing, J. R. Diffusion-Edited NMR − Affinity NMR for Direct Observation of Molecular Interactions. *J. Am. Chem. Soc.* **1997**, *119*, 5249−5250.
(7) Hajduk, P. J.; Olejniczak, E. T.; Fesik, S. W. One-Dimensional Relaxation- And Diffusion-Edited NMR Methods For Screening Compounds That Bind To Macromolecules. *J. Am. Chem. Soc.* **1997**, *119*, 12257−12261.
(8) Meyer, B.; Weimar, T.; Peters, T. Screening Mixtures for Biological Activity by NMR. *Eur. J. Biochem.* **1997**, *246*, 705−709.
(9) Chen, A.; Shapiro, M. J. NOE Pumping: A Novel NMR Technique for Identification of Compounds with Binding Affinity to Macromolecules. *J. Am. Chem. Soc.* **1998**, *120*, 10258−10259.
(10) Mayer, M.; Meyer, B. Characterization of Ligand Binding by Saturation Transfer Difference NMR Spectroscopy. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1784−1788.
(11) Hajduk, P. J.; Sheppard, G.; Nettesheim, D. G.; Olejniczak, E. T.; Shuker, S. B.; Meadows, R. P.; Steinman, D. H.; Carrera, G. M.; Marcotte, P. A.; Severin, J.; Walter, K.; Smith, H.; Gubbins, E.; Simmer, R.; Holzman, T. F.; Morgan, D. W.; Davidsen, S. K.; Fesik, S. W. Discovery of Potent Nonpeptide Inhibitors of Stromelysin Using SAR by NMR. *J. Am. Chem. Soc.* **1997**, *119*, 5818−5827.
(12) Hajduk, P. J.; Dinges, J.; Miknis, G. F.; Merlock, M.; Middleton, T.; Kempf, D. J.; Egan, D. A.; Walter, K. A.; Robins, T. S.; Shuker, S. B.; Holzman, T. F.; Fesik, S. W. NMR−Based Discovery of Lead Inhibitors that Block DNA Binding of the Human Papillomavirus E2 Protein. *J. Med. Chem.* **1997**, *40*, 3144−3150.
(13) Hajduk, P. J.; Dinges, J.; Schkeryantz, J. M.; Janowick, D.; Kaminski, M.; Tufano, M.; Augeri, D. J.; Petros, A.; Nienaber, V.; Zhong, P.; Hammond, R.; Coen, M.; Beutel, B.; Katz, L.; Fesik, S. W. Novel Inhibitors of Erm Methyltransferases from NMR and Parallel Synthesis. *J. Med. Chem.* **1999**, *42*, 3852−3859.
(14) Hajduk, P. J.; Zhou, M.-M.; Fesik, S. W. NMR−Based Discovery Of Phosphotyrosine Mimetics That Bind To The Lck SH2 Domain. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2403−2406.
(15) Fairbrother, W. J.; Champe, M. A.; Christinger, H. W.; Keyt, B. A.; Starovasnik, M. A. ^{1}H, ^{13}C, and ^{15}N Backbone Assignment and Secondary Structure of the Receptor Binding Domain of Vascular Endothelial Growth Factor. *Protein Sci.* **1997**, *6*, 2250−2260.
(16) Gilman, A. G.; Rall, T. W.; Mies, A. S.; Taylor, P. *The Pharmaceutical Basis of Therapeutics*, 8th ed.; McGraw-Hill, Inc.: NewYork, 1993.
(17) McGaughey, G. B.; Gagne, M.; Rappe, A. K. π-Stacking Interactions. Alive and Well in Proteins. *J. Biol. Chem.* **1998**, *273*, 15458−15463.
(18) van der Spoel, D.; van Buuren, A. R.; Tieleman, D. P.; Berendsen, H. J. C. Molecular Dynamics Simulations of Peptides from BPTI: A Closer Look at Amide-Aromatic Interactions. *J. Biomol. NMR* **1996**, *8*, 229−238.
(19) Sapse, A.-M.; Schweitzer, B. S.; Dicker, A. P.; Bertino, J. R.; Frecer, V. *Ab Initio* Studies of Aromatic−Aromatic and Aromatic-Polar Interactions in the Binding of Substrate and Inhibitor to Dihydrofolate Reductase. *Int. J. Pept. Protein Res.* **1992**, *39*, 18−23.
(20) Mecozzi, S.; West, A. P., Jr.; Dougherty, D. A. Cation-π Interactions in Aromatics of Biological and Medicinal Interest: Electrostatic Potential Surfaces as a Useful Qualitative Guide. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10566−10571.

(21) Scrutton, N. S.; Raine, A. R. C. Cation-π Bonding and Amino-Aromatic Interactions in the Biomolecular Recognition of Substituted Ammonium Ligands. *Biochem. J.* **1996**, *319*, 1−8.
(22) Pavia, M. R.; Cohen, M. P.; Dilley, G. J.; Dubuc, G. R.; Durgin, T. L.; Forman, F. W.; Hediger, M. E.; Milot, G.; Powers, T. S.; Sucholeiki, I.; Zhou, S.; Hangauer, D. G. The Design and Synthesis of Substituted Biphenyl Libraries. *Bioorg. Med. Chem.* **1996**, *4*, 659−666.
(23) *Physicians' Desk Reference*; Medical Economics Company, Inc.: Montvale, NJ, 2000.
(24) Wolfe, S. A.; Zhou, P.; Dotsch, V.; Chen, L.; You, A.; Ho, S. N.; Crabtree, G. R.; Wagner, G.; Verdine, G. L. Unusual Rel-Like Architecture in the DNA-Binding Domain of the Transcription Factor NFATc. *Nature* **1997**, *385*, 172−176.
(25) Ranganathan, R.; Lu, K. P.; Hunter, T.; Noel, J. P. Structural and Functional Analysis of the Mitotic Rotamase Pin1 Suggests Substrate Recognition is Phosphorylation Dependent. *Cell* **1997**, *89*, 875−886.
(26) Sattler, M.; Liang, H.; Nettesheim, D.; Meadows, R. P.; Harlan, J. E.; Eberstadt, M.; Yoon, H. S.; Shuker, S. B.; Chang, B. S.; Minn, A. J.; Thompson, C. B.; Fesik, S. W. Structure of Bcl-X(L)-Bak Peptide Complex − Recognition Between Regulators of Apoptosis. *Science* **1997**, *275*, 983−986.
(27) Medek, A.; Petros, A. M.; Nettesheim, D. G.; Kim, D.; Yoon, H. S.; Meadows, R. P.; Fesik, S. W. NMR Solution Structure of Human Protein Bcl-2, an Inhibitor of Programmed Cell Death. *Manuscript in preparation*.
(28) Barford, D.; Flint, A. J.; Tonks, N. K. Crystal Structure of Human Protein Tyrosine Phosphatase 1B. *Science* **1994**, *263*, 1397−1402.
(29) Kay, L. E.; Keifer, P.; Saarinen, T. Pure Absorption Gradient Enhanced Heteronuclear Single Quantum Correlation Spectroscopy with Improved Sensitivity. *J. Am. Chem. Soc.* **1992**, *114*, 10663−10665.
(30) McCullagh, P.; Nelder, J. A. *Generalized Linear Models*; Chapman and Hall: New York, 1996.

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