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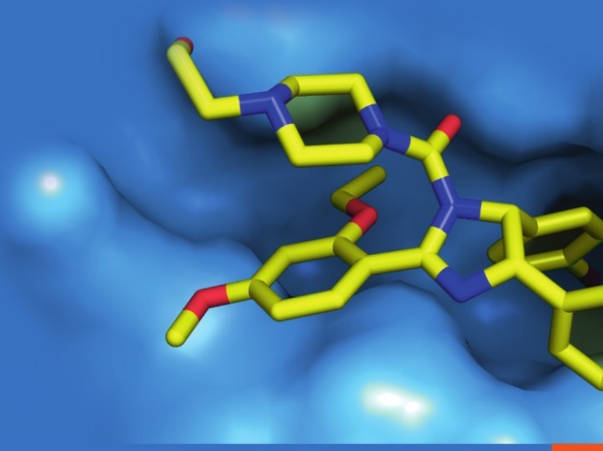

Lyubomir Vassilev
David Fry
Editors

Small-Molecule Inhibitors of Protein-Protein Interactions

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Preface

Cell integrity and functions depend on a myriad of protein–protein interactions. Many of these interactions are involved in disease etiology and represent desirable targets for pharmacological intervention. However, the notion of modulating protein–protein binding with small molecules has historically raised serious concerns. The interface between two interacting proteins is typically large and devoid of sizable subpockets. It has been thought unlikely for a drug-like molecule to bind to such a landscape with high affinity and to effectively compete away one of the protein partners. However, this blanket characterization of protein–protein interfaces is overly simplistic. It has become clear that in certain cases reasonably sized pockets exist to support binding, or that in other cases the interface region is flexible and an incoming molecule can induce the formation of a suitable binding pocket. On the other side of the issue, the concept of what constitutes a drug-like molecule has been evolving, particularly in the context of protein–protein modulators. The traditional profile of an organic compound with a molecular weight in the 200–500 range has been expanded to include compounds of significantly higher molecular weight, and the possibility of using peptides and peptide-like molecules as drugs has become much more realistic.

In recent years, several success stories have appeared with regard to discovery of protein–protein interaction inhibitors. There is a growing understanding of the critical factors involved and of the fundamental issues relating to the many aspects of the process – choosing targets, finding leads, discerning and verifying binding strategies, and optimizing properties. In this volume, we have collected the knowledgeable insights of a number of leaders in this field – researchers who have achieved success in addressing the difficult problem of inhibiting protein–protein interactions. They describe their unique approaches and share experiences, results, thoughts, and opinions. The content of the chapters is rich, and in terms of scope ranges from generalized approaches to specific case studies. There are various focal points, including methodologies and the molecules themselves. Ultimately, there are numerous lessons to be taken away from this collection, and we hope that this snapshot of the current state of the art in developing protein–protein inhibitors

not only pays tribute to the past successes but also generates excitement about the future potential of this field.

Nutley, New Jersey

Lyubomir Vassilev
David Fry

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Hydrogen-Bonded Synthetic Mimics of Protein Secondary Structure as Disruptors of Protein–Protein Interactions

Marc J. Adler, Andrew G. Jamieson, and Andrew D. Hamilton

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Abstract Small molecules which can mimic the key structural facets of protein secondary structure, in particular the α-helix, β-strand, and β-sheet, have been shown to be potent disruptors of protein–protein interactions. Researchers have recently taken the organizational imitation of protein secondary structure to a new level by using intramolecular hydrogen bonds as stabilizing forces in these small molecule mimetics. The inclusion of these interactions invokes a conformational bias of the system, allowing for greater control of the appearance, and thus often function, of these molecules by design.

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1 Introduction

The interaction of two proteins represents an important process for naturally mediating cellular function. While these interactions often involve large surface areas coming into contact with each other, it is often just a few small "hot spots" which invoke the specificity and effectiveness of such binding events. On a molecular level, the nature of these interactions is noncovalent, including hydrogen bonding, hydrophobic, ionic, pi-stacking, and van der Waals contacts. Furthermore, the spatial orientation of these "hot spots" is often the result of defined secondary structures, including α-helices, β-strands, and β-sheets; the specific angular projection and interfunctionality distance imposed by these scaffolds are responsible for the recognition element of the binding event.

Small molecules that are able to discriminately interrupt interactions of this type have been hotly pursued for myriad purposes ranging from investigational tools in the laboratory to potential therapeutic agents. One approach that researchers have taken toward protein–protein interaction inhibition is to design small molecules that mimic the structure, and thus the function, of the crucial elements of secondary structural motifs.

Many strategies have been used for the creation of proteomimetic small molecules, and this topic has been reviewed extensively (Ross et al. 2010; Saraogi and Hamilton 2008; Davis et al. 2007; Loughlin et al. 2004; Schneider and Kelly 1995; Nesloney and Kelly 1996; Glenn and Fairlie 2002; Nowick 2006, 2008; Wilson 2009; Fuller et al. 2009). Of particular interest within this topic, however, are proteomimetic molecules designed to use the same stabilizing force that the proteins themselves use: intramolecular hydrogen bonding. The incorporation of this structural facet provides the molecules not only a level of organizational elegance and intricate nature mimicry, but also often increased functionality.

This review covers the field of small molecule protein secondary structure mimics which both possess structurally relevant hydrogen bonds and aspire to modulate protein–protein interactions.

2 α-Helix Mimicry

The peptide α-helix is the most commonly observed secondary structure (Fig. 1). It is stabilized by an extensive hydrogen bonding network, whereby each amide carbonyl oxygen of residue *i* is engaged in a hydrogen bond to the amide NH proton of the $i + 4$ residue. Each turn of the helix (i.e., helical pitch) covers 5.4 Å and is composed of approximately 3.6 amino acids. All the amino acid side chains are projected on the outer face of the helix.

A peptide that is composed of the amino acids of a helix-forming region of a peptide will only rarely spontaneously form an α-helix on its own; further stabilization from the rest of the protein assists in the formation of this secondary

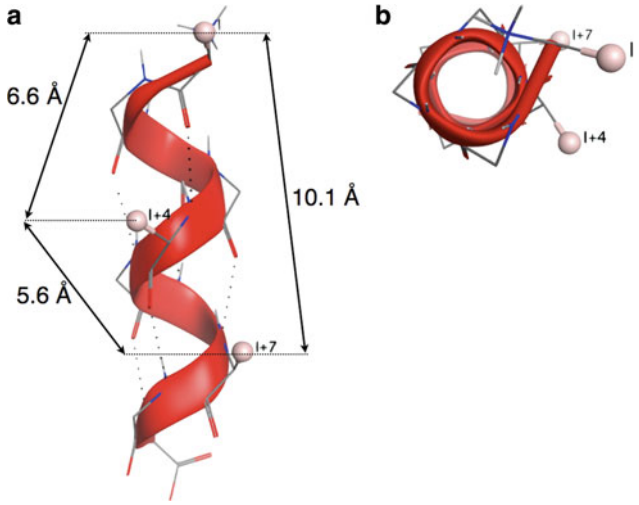

Fig. 1 Views of an idealized α-helix from the *front* (**a**) and *top* (**b**) with respect to the *i*, *i+4*, and *i+7* residues, relevant side chains for single-face mimicry

structure. This means that for researchers, biologically relevant helical segments of proteins cannot simply be excised from their parent protein and used in a clinical setting. This fact is the primary driving force behind the desire to create small molecule α-helix mimics.

In natural systems, α-helices mediate interactions via their side chains, as the backbone is tied up in the hydrogen-bonding network. Therefore, a molecule which seeks to act as a helix mimic must imitate the spatial orientation of the side groups being projected on one or more faces of the α-helix in order to replicate the recognition motif.

The investigation of synthetic α-helicomimetic molecules for the purpose of inhibiting protein–protein interactions is a field of research that has recently been quite active (Ross et al. 2010; Saraogi and Hamilton 2008; Davis et al. 2007). Peptidic variants, such as "stapled" peptides, α/β-peptide hybrids, and peptoids, have been synthesized in a number of laboratories and shown to indeed mimic the structure and often function of naturally occurring helices. Some of these structures, notably the α/β-peptide hybrids, do in fact use hydrogen bonds extensively for secondary structure stabilization. In addition, many nonpeptidic small molecules (including indanes, terphenyls, terpyridines, and pyridazines) have been used successfully for the mimicry of α-helices. These scaffolds, however, are not structurally influenced by hydrogen bonds.

A simple, illustrative example of this type of scaffold is the oligobenzamide structure (Figs. 2 and 3). In this motif, the amide NH proton engages in a core-stabilizing hydrogen bond with a lone pair of electrons from the oxygen of the aryl-alkoxy group. Depending on the substitution pattern, this noncovalent interaction completes either a five- (Fig. 2) or six-membered ring (Fig. 3). Evidence for the

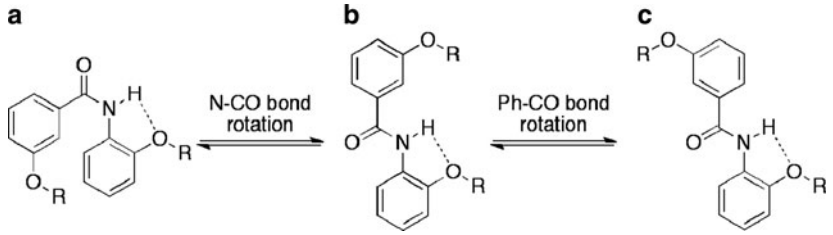

Fig. 2 Rotamers of the oligobenzamides maintaining the five-membered hydrogen-bonded ring

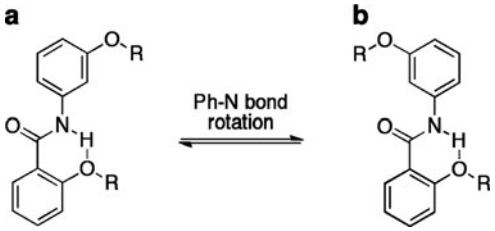

Fig. 3 Rotamers of the oligobenzamides maintaining the six-membered hydrogen-bonded ring

presence of this organizational hydrogen bond can be found in a number of crystal structures, which show the O–H interatomic distances to be less than the sum of their van der Waals radii in both cases.

For a molecule to mimic one face of an α-helix, the side groups must all be projected on the same side of the scaffold. Ideally, oligobenzamides would prefer to be completely planar, thus giving each bond in the phenyl-*N*-carbonyl-phenyl series two distinct, local energy-minimized states.

In the simple benzamides, a five-membered hydrogen-bonded ring (formed when the alkoxy substituents are *ortho* to the amide N) effectively dictates the state of the phenyl-N bond, as in only one of the conformations can the hydrogen bond be successfully engaged (Fig. 2). The N-carbonyl bond naturally prefers the s-*trans* (with regard to the benzene rings) state (Fig. 2b) in order to minimize steric clashing between the two large phenyl groups (Fig. 2a). The carbonyl-phenyl bond, however, does not have a preference with regard to its orientation. While molecular modeling of this compound suggests that it prefers to orient the side groups opposite from each other (Fig. 2c), NMR studies (Plante et al. 2008) show that both states exist in solution. The fact that molecules of this type are able to successfully mimic α-helices gives credence to the idea that these molecules are able to access states where the side groups are projected on the same face of the oligomer (Fig. 2b).

In the case of the six-membered hydrogen-bonded ring (where the alkoxy substituents are *ortho* to the carbonyl), this noncovalent interaction defines both the phenyl-carbonyl and the carbonyl-N configuration. This leaves only the N-phenyl bond with free rotation, relatively speaking (Fig. 3); while only one of these conformations leads to helix mimicry, both states can exist in solution.

2.1 Hamilton's Oligopyridylamides

The first instance of small molecule α-helix mimicry by hydrogen-bonded scaffolds, reported in 2003 by Hamilton et al., used an oligopyridylamide scaffold (Fig. 4a) (Ernst et al. 2003). These compounds are stabilized not only by an alkoxy-O/amide-NH intramolecular hydrogen bond, but also by the same proton noncovalently interacting with the nitrogen of the pyridine ring (Fig. 4a). A crystal structure obtained of a trimer revealed the presence of both of these hydrogen bonds in the solid phase, and variable temperature ^{1}H NMR experiments verified these interactions in both polar (DMSO-d_6) and nonpolar (CD_2Cl_2) solvents.

The synthesized trimeric and tetrameric molecules were also used to demonstrate the utility of this scaffold to design potentially promising anticancer agents via inhibition of the Bak BH3/Bcl-xL interaction. The examination of these molecules using a fluorescence polarization (FP) assay showed that α-helix mimics of this type could competitively displace a fluorescein-labeled Bak BH3 peptide from its hydrophobic binding spot on Bcl-xL with potencies in the low-micromolar range.

Researchers in the Hamilton lab used this scaffold to make helicomimetic molecules, which could, depending on the experimental conditions, either agonize or antagonize the aggregation of islet amyloid polypeptide (IAPP) (Saraogi et al. 2010; Hebda et al. 2009), a process believed to be involved in the pathology of type II diabetes. Specifically, the misfolding and aggregation of IAPP have been clearly linked with the cell death of insulin-secreting β-cells.

Oligopyridylamide oligomers of varying length bearing oxymethylenecarboxy substituents (Fig. 4b) were synthesized and shown to initiate IAPP aggregation in the absence of a lipid bilayer (which normally catalyzes fiber formation); in the

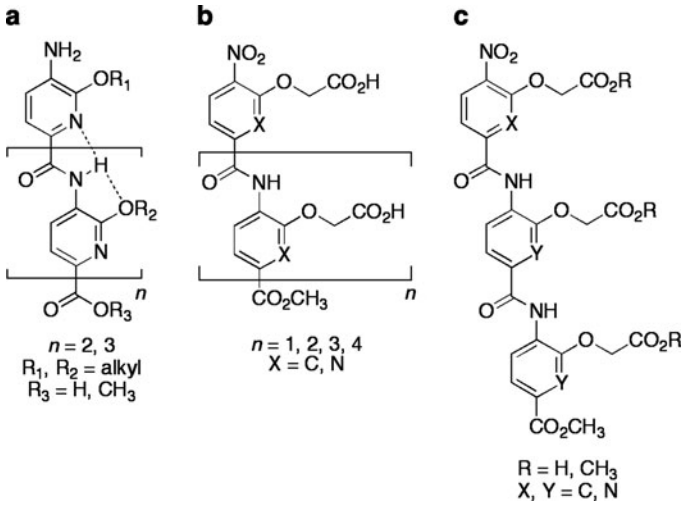

Fig. 4 α-helix mimetic oligopyridylamides from the Hamilton lab

presence of the lipid bilayer, however, the same molecule acts as an inhibitor of the lipid bilayer-catalyzed IAPP fiber formation and has been shown to reduce IAPP-induced cytotoxicity in a well-characterized β-cell model.

In addition, an investigation into the structural ramifications of the movement from oligobenzamides to oligopyridylamides (Fig. 4c) was launched by the Hamilton lab (Saraogi et al. 2008). Crystal structures showed clearly that the presence of the extra five-membered ring forming hydrogen bond between the pyridinyl nitrogen and the amide NH proton induced a curvature to the backbone. This effect was quantified based on serial replacement of the backbone benzenes with pyridines; as expected, more pyridines (yielding more five-membered hydrogen-bonded rings) increased the curvature of the oligomers.

2.2 *Oligobenzamides*

Of the nonpeptidic scaffolds that use an intramolecular hydrogen bond, the oligobenzamide structure (Figs. 2 and 3) has been the most prevalent (Plante et al. 2008, 2009; Ahn and Han 2007; Marimganti et al. 2009; Shaginian et al. 2009). The most fundamental example of this scaffold can be seen in work from the labs of Wilson (Plante et al. 2008, 2009) and Ahn (Ahn and Han 2007; Marimganti et al. 2009). The oligobenzamides were generally synthesized in an iterative form, where coupling occurred via the reaction of a free aniline with a benzoic acid derivative.

Wilson et al. synthesized oligobenzamides (Fig. 5a) which could mimic up to five turns of an α-helix (Plante et al. 2008). A crystal structure of the trimer was obtained, with amide-H/alkoxy-O interatomic distances of 2.155 and 2.132 Å. Evidence for the existence of these intramolecular hydrogen bonds in solution was given using NMR, as the amide protons do not display any change in chemical shift upon dilution in either $CDCl_3$ or DMSO-d_6, while significantly different

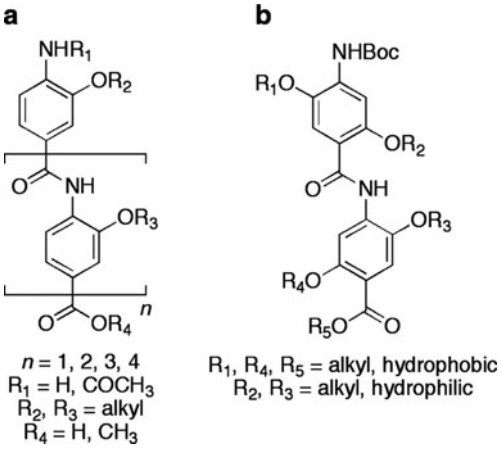

Fig. 5 (**a**) Oligobenzamide α-helix mimetic scaffold used by Wilson and Ahn. (**b**) Ahn's amphiphilic α-helix mimetic scaffold

temperature-induced shifts are observed in DMSO-d_6 and CD_2Cl_2. They observed via ^{1}H-^{1}H NOESY that both rotamers of the carbonyl–phenyl bond exist in solution; interestingly, a crystal structure of a trimer shows two of the side groups being projected onto the same face, while the third is rotated to the opposite side.

Wilson and coworkers later used derivatives of this scaffold to inhibit the tumor protein 53 (p53)–human double minute-2 oncogene (hDM2) interaction (Plante et al. 2009). This target, which features the interaction of a helical section of the p53 peptide with a hydrophobic cleft on the surface of hDM2, has often been exploited to demonstrate the therapeutical application of α-helix mimetic molecules. In cancerous cells, hDM2 is overexpressed and subsequently binds to p53; this event prevents p53 from performing its role in initiating apoptosis and thus suppressing tumor growth. Inhibition of this protein–protein interaction has been shown to be an effective anticancer chemotherapeutic approach (Vassilev et al. 2004).

In a heroic effort of synthetic endeavor, Boger et al. assembled and examined myriad oligoamides for activity in inhibiting the hDM2/p53 interaction (Shaginian et al. 2009). After initial survey of 80 molecules based on a multitude of scaffolds (Fig. 6), they determined that a single benzene ring *para*-substituted by two single amino acids via amide bonds (Fig. 6d) was the most promising in vitro α-helix mimics for their library. Using this scaffold, they then synthesized a library of 400 mixtures of 20 unique compounds each (20 amino acids × 20 alkoxyaminobenzoates × 20 amino acids) and found that residues containing a central alkoxyethyleneindole performed best in their assay. Further deconvolution was performed by resynthesizing the individual 20 members of the best performing 20-compound

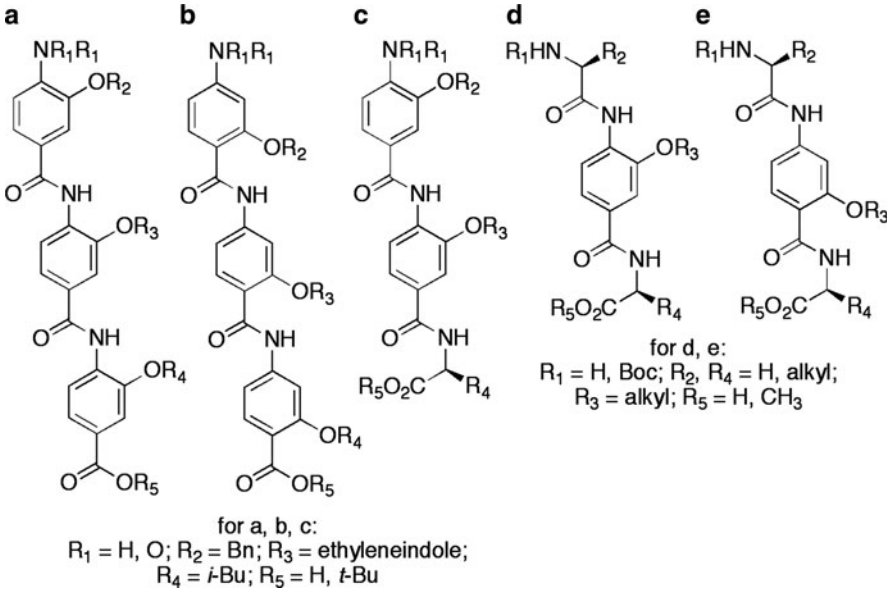

Fig. 6 Scaffolds used for α-helix mimicry by the Boger lab