Synthetic non-peptide mimetics of α-helices

Jessica M. Davis,[a] Lun K. Tsou[b] and Andrew D. Hamilton*[b]

Received 11th September 2006
First published as an Advance Article on the web 4th January 2007
DOI: 10.1039/b608043j

Proteins in nature fold into native conformations in which combinations of peripherally projected aliphatic, aromatic and ionic functionalities direct a wide range of properties. α-Helices, one of the most common protein secondary structures, serve as important recognition regions on protein surfaces for numerous protein–protein, protein–DNA and protein–RNA interactions. These interactions are characterized by conserved structural features within the α-helical domain. Rational design of structural mimetics of these domains with synthetic small molecules has proven an effective means to modulate such protein functions. In this *tutorial review* we discuss strategies that utilize synthetic small-molecule antagonists to selectively target essential protein–protein interactions involved in certain diseases. We also evaluate some of the protein–protein interactions that have been or are potential targets for α-helix mimetics.

1 Introduction

Specific protein–protein complexes arising from the interactions of protein surface regions are one of the ways that Nature achieves control and function within the complexity of a living cell. Development of strategies to selectively disrupt such protein–protein interactions is of paramount importance. Successful disruption of protein function by designed molecules will validate our understanding of their physicochemical properties and their relationship to certain disease pathways. The macromolecular mimetic approach involves the preparation of molecules with controlled molecular dimensions to target protein surface functionalities and is relatively new in medicinal chemistry. In proteins, these surfaces often contain critical amino acid residues within domains known as ''hot spots''. By mimicking the interactions within these domains using synthetic molecules, we can regulate protein function to affect biological processes. The biomolecular mimetic approach to therapeutic design has recently been comprehensively reviewed elsewhere.[1] We herein review key protein–protein interactions that are mediated by α-helices and the development of mimetics that target such interactions.

[a]*Department of Chemistry, Fairfield University, 1073 North Benson Rd, Fairfield, CT 06824, USA. E-mail: jmdavis@mail.fairfield.edu; Tel: 203-254-4000 ext 2123*
[b]*Department of Chemistry, Yale University, 225 Prospect St. New Haven, CT 06511, USA. E-mail: lun.tsou@yale.edu; Andrew.hamilton@yale.edu; Fax: 203-432-3221; Tel: 203-432-5570*

2 α-Helix interactions and the mimetic approach to drug design

2.1 α-Helices

α-Helices make up the majority of secondary protein structure and comprehensive reviews on their occurrence in proteins can be found elsewhere.[2] However, specific features of the α-helix are important in the consideration of factors involved in the design of mimetics.

In globular proteins, α-helices make up about a third of protein secondary structure and tend to be at least 10 residues long (3 turns). Statistical analysis has shown that variations in

Jessica M. Davis

Jessica M. Davis is an assistant professor of chemistry at Fairfield University. Her current research involves targeting autoimmune disease through a protein mimetic approach. She received her PhD in 2006 from Yale University under the direction of Professor Andrew D. Hamilton. Her PhD work involved the development of α-helix mimetics based on novel scaffold designs.

Lun K. Tsou

Lun K. Tsou earned a BS degree in Chemistry with Honors from the University of North Carolina at Chapel Hill in 2002. His undergraduate research was under the supervision of Professor Marcey L. Waters to investigate the energetics of cation–π interactions in aqueous media using α-helix models. Currently, under the direction of Professor Andrew D. Hamilton, his research at Yale involves the development of protein surface receptors based on macrocyclic scaffolds for modulation of protein–protein interactions.

helix geometry are related to the amino acid sequence, with about 15% being linear, 20% kinked, and 60% curved.[3] The helix is often associated with blocks of hydrophobic residues (consisting of Ala, Ile, Leu, Met, Phe, Pro, Trp, Tyr, and Val) under 16 amino acids long.[4] Non-polar residues, including Ala, Leu, Val, and Ile are preferred in the i, $i + 1$, $i + 4$, and $i + 5$ positions with various amino acids in other positions, save Pro, which normally occurs only at the terminus.[5]

Transmembrane proteins consist mostly of α-helical domains and the sequence motifs are more defined than in globular proteins. Studies have revealed over-represented motifs from statistical predictions that are conserved in transporter, symporter, and channel forming protein families. These motifs are characterized by 2 small amino acids (Gly, Ala, or Ser) and two large amino acids (Ile, Val, or Leu) in the i, $i + 4$ positions or by one large and one small in the i, $i + 1$ positions. Combinations of these motifs also exist with small, large, small in the i, $i + 1$, and $i + 4$ positions.[2] In particular, GlyGly4, IleIle4, GlyAla4, and IleGly1 are over-represented from statistical predictions.[6]

Transmembrane helix–helix interactions occur at various packing angles between -56° and 67°, with the left-handed angles between 15° and 20° being most favored. In transmembrane proteins, the interface is mediated by the aforementioned motifs and stabilized by weak Cα–H···O hydrogen bonds which are made possible by small residues at the interface. These small residues also allow for changes in conformation necessary for protein function. In cases where these small residues are not found, motifs such as SxxxSSxxT create hydrogen bonding networks at helix–helix interfaces.[2] The packing of α-helices seen in globular proteins is less definable because of the diversity of these proteins and is characterized by one face often being hydrophilic and exposed to the solvent and the opposite face being hydrophobic and involved in protein folding or protein–protein interactions.[5]

Andrew D. Hamilton

Andrew D. Hamilton received his PhD from Cambridge University in 1980, and the following year carried out his postdoctoral research under the direction of Professor Jean-Marie Lehn at Université Louis Pasteur, Strasbourg. In the fall of 1997, he moved to Yale University where he is now the Benjamin Silliman Professor of Chemistry and the Provost of the university. In 2004, he was elected as a Fellow of the Royal Society. The principal research interest of Professor Hamilton is in the field of molecular recognition and its application to problems in organic and biological chemistry. Approaches include using synthetic molecules for artificial receptor design, peptidomimetic design, proteomimetic design, and modulation of signal transduction through the disruption of protein–protein interactions.

3 Synthetic α-helix mimetics

3.1 *i*, *i* + 1 mimetics

Indanes. Researchers at Parke-Davis have shown that a 1,6-disubstituted indane mimics the i, $i + 1$ residues of an α-helix.[7–9] Of the two enantiomers of the designed skeleton, only the S-isomer mimics the α-helix. Computer modeling studies show a root mean square deviation of 0.2 Å for the 1,6-substituents superimposed on the Cα, Cβ carbon atoms of the helix residues i, $i + 1$. Moreover, the second substituent on the 1 position (labeled M) potentially allows mimicry of the $i - 1$ residue of an α-helix (Fig. 1).

3.2 *i*, (*i* + 3), *i* + 4, *i* + 7… mimetics

Terphenyl. Hamilton and coworkers have developed mimetics of the hydrophobic face of an α-helix using a terphenyl scaffold.[10,11] A tris-*ortho*-substituted terphenyl can mimic the i, $i + 4$, and $i + 7$ residues of the α-helix by adopting a staggered conformation that closely reproduces the angular orientation of the peripheral functionalities on the helical surface. To increase the water solubility of the design, a heteroatom-based terpyridine scaffold was developed analogously to the terphenyl to mimic the i, $i + 4$, and $i + 7$ residues of an α-helix.[12] Similar structural mimicry is expected as the staggered conformation is retained within the terpyridine backbone (Fig. 2).

Many helix/protein complexes exploit additional interactions deriving from residues flanking the hydrophobic face of the helix. Recently, a diphenylindane-based proteomimetic has been developed by Kim and Hamilton to reproduce the projection of i, $i + 3$, $i + 4$, and $i + 7$ residues on the α-helix.[13] MM2 calculations on tetramethyl-substituted 4,7-diphenylindanes revealed that aryl–aryl torsion angles of 62° and 67° lead to an angular projection of the four substituents that closely mimics those on one face of a helix with a RMSD difference of 0.92 Å (Fig. 2).

In order to increase solubility and ease of synthesis of the mimetics, Hamilton *et al*. have also developed terephthalamide-based scaffolds.[14] The flanking phenyl rings in the terphenyl scaffold were replaced by two functionalized carboxamide groups. A conformational constraint in the scaffold derives from an intramolecular hydrogen bond between the amide –NH and the alkoxy oxygen atom which then influences the projection of the R4 side chain. Calculations using the QikProp program suggested that terephthalamide has a log*P* value (partition coefficient for n-octanol/water) of 4.42, compared to 9.25 for that of the terphenyl, indicating that terephthalamide derivatives should have improved water

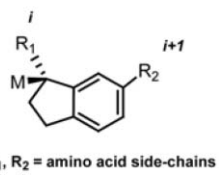

Fig. 1 The indane template mimicking an α-helix, showing the i, $i + 1$ positions.

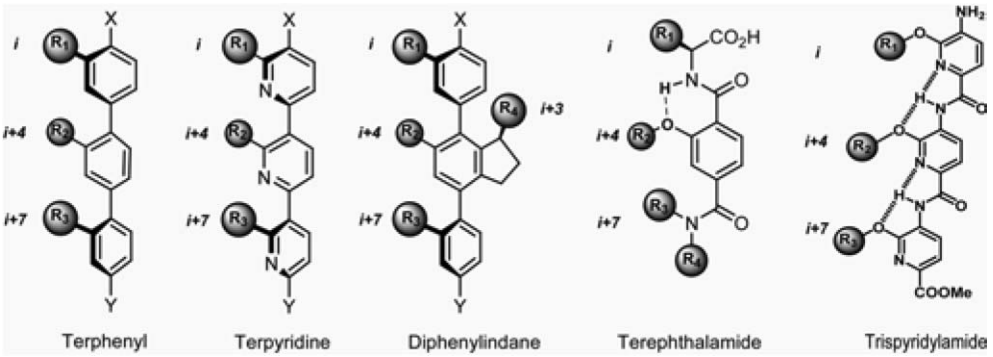

Fig. 2 α-Helix mimetics developed by Hamilton and coworkers. The positions shown are structural mimics of *i*, *i* + 3, *i* + 4 and *i* + 7 residues of the helix.

solubility and membrane permeability. Superimposition of the preferred conformation (from MM2 calculation) of the terephthalamide substituents on the *i*, *i* + 4 and *i* + 7 side chains of an α-helix gave an RMSD value of 1.03 Å (Fig. 2).

Oligophenyls. Matile *et al*. have designed and synthesized novel *para*-oligophenyls as α-helix mimetics.[15] The elongated *p*-octaphenyls adopt an extended conformation to mimic the backbone of the helix, with the functionalities projected from the *ortho*/*meta*-positions of the phenyl rings mimicking the helix side-chains. Systematic substitutions of the substituents allow the study of the role of backbone dipole as well as side chain interactions and the opportunity for mimicking more sophisticated protein structures on both the structural and functional level.

Benzylideneacetophenones (chalcones). Stoll *et al*. used rational design to develop α-helix mimetics based on chalcones, some of which show antitumor activity.[16] ^{15}N-HSQC NMR spectroscopy was used to investigate the possible binding mode of chalcone derivatives with the target protein MDM2. Certain chalcones were shown to disrupt the binding of MDM2 with p53, a known α-helical interaction. The NMR analysis showed that chalcone C (Fig. 3) adopted an extended conformation at the binding interface with a positioning of substituents that is similar to the *i*, *i* + 4, *i* + 7 groups on p53.

***trans*-Fused polycyclic ethers.** Oguri and co-workers recently have reported a strategy for the topological mimicry of an α-helix with synthetic *trans*-fused polycyclic ethers that are conceptually derived from marine toxins.[17] Efficient synthesis afforded the stereo-controlled construction of the *trans*-fused polycyclic ether skeleton through SmI_2-mediated Reformatsky-type coupling of α-sulfonyl ketones with aldehydes. The ladder-like 6/6/6 tricyclic ether system consists of consecutive skeletal oxygen atoms on the same side separated by a distance of 4.8 Å. This distance is almost identical to the interval (*ca*. 5 Å) between the *i* and *i* + 4 side-chains in an α-helix (Fig. 3).

1,4-Benzodiazepine-2,5-diones (BZD). Cummings and co-workers reported the use of 1,4-benzodiazepine-2,5-diones (BZD) as mimetics of the *i*, *i* + 4 and *i* + 7 positions on an α-helix (Fig. 3).[18] Through screening a compound collection for antagonists of the HDM2–p53 interaction (see section 5), BZD was identified. Based on conformational analysis and molecular docking, derivatives of the BZD scaffold were designed and synthesized to mimic the *i*, *i* + 4 and *i* + 7 side-chains of the hydrophobic face of an α-helix (p53). The crystal structure of BZD bound to HDM2 provides evidence that the scaffold indeed mimics the *i*, *i* + 4 and *i* + 7 positions of the natural peptide ligand.

Trisubstituted imidazole. Antuch and co-workers employed a modular parallel synthesis using multicomponent reaction (MCR) to structurally convert Hamilton's terphenyl scaffold into an easily accessible trisubstituted imidazole.[19] Modeling studies showed that trisubstituted imidazole derivatives, obtained *via* easy and versatile van Leusen MCR, could effectively mimic *i*, *i* + 3 and *i* + 7 positions of an α-helix (Fig. 3).

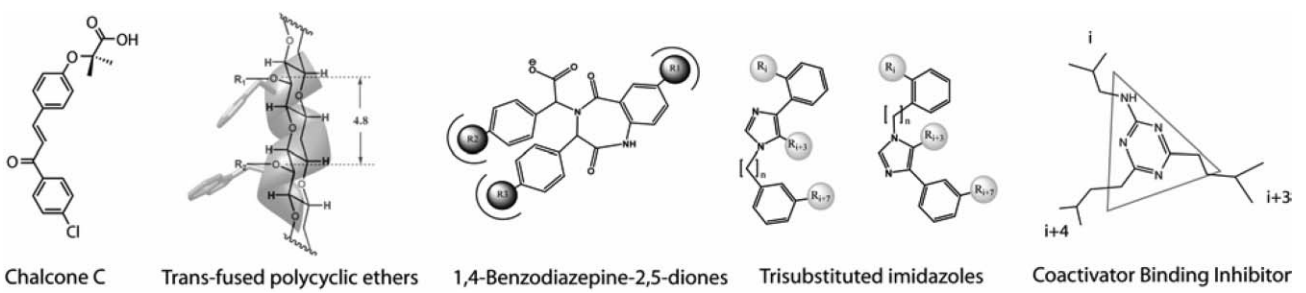

Fig. 3 Synthetic small molecules used as α-helix mimetics by various groups.

3.3 *i*, *i* + 3, *i* + 4 mimetics

Coactivator binding inibitors (CBI). Rodriguez *et al.* used a rational design approach to create small molecule mimetics of the LXXLL motif found in many nuclear receptor–coactivator interfaces.[20] Small molecule inhibitors of coactivator binding were designed following the ''outside-in'' strategy. This approach begins with a mimic for the helix backbone, which is outside of the hydrophobic binding groove, and then proceeds by adding elements of the key residues. (see section 4.4) A head-on view of the coactivator peptide shows that the positions of the three leucine residues in the LXXLL sequence fall roughly into the shape of an equilateral triangle. The strategy was then to employ a central core with the correct dimensions to position hydrophobic substituents that mimic the three leucine residues of the coactivator peptide in an *i*, *i* + 3, *i* + 4 relationship (Fig. 3).

4 Targets for α-helix mimetics that inhibit protein–protein interactions

Protein–protein interactions that involve α-helices are numerous and many are involved in pathways that are critical in certain human diseases.[21,22] As a result, a number of these interactions represent important therapeutic targets using the α-helix mimetic approach and have been the focus of considerable attention (Table 1).

4.1 Transmembrane proteins

A number of membrane transporters exist with varying functions and mechanisms. In general, they create membrane pores with hydrophilic cores and hydrophobic exteriors that make favorable contact with the lipophilic membrane. Membrane transporters that operate through passive mediated transport allow the diffusion of molecules from a region of high concentration to one of low concentration. In contrast, pores that function through active transport allow the endergonic process of moving molecules from a low concentration region to one of high concentration by coupling the transport with an exergonic process. Three examples of membrane transporters involving key α-helical interactions are discussed below.

Bacterial autotransporter NaIP. The Gram negative bacterial autotransporter NaIP from *Neisseria meningitides* is used to transport proteins across the outer membrane. Various mechanisms for the translocation of proteins *via* NaIP have been proposed and the crystal structure of the protein has provided some insight.[23] The crystal structure reveals a β-barrel with an N-terminal α-helix functionality to block the entry. One face of the α-helix makes hydrophobic and hydrophilic contacts, including salt bridges and H-bonds, with the interior of the β-barrel, which is predominately made up of hydrophilic residues. A small channel between the α-helix and the barrel wall allows the simple diffusion of salts and it has been proposed that increasing the salt concentration results in displacement of the α-helix. This displacement in turn permits the uptake of larger molecules, including antibiotics. Elimination of this N-terminal α-helix increases pore activity as well as antibiotic uptake. The interacting residues on the helix include Asp792, Asp799, Arg803, Lys806, Asp810, and Asp813: a repeating *i*, *i* + 4, *i* + 7 motif.

In direct analogy to this process, Matile *et al.* have designed rigid oligophenylene scaffolds with peripheral pentapeptide substituents (Fig. 4).[15,24] Through hydrogen bonding and β-sheet formation along the peptide strands, these molecules are capable of aggregating to form a tetrameric analog of the pore channel. In turn, related oligophenylenes can function like the α-helix domain in NaIP and insert into the artificial barrel blocking the transport of species through the center (Fig. 4).

Gp41. Gp41 is a membrane transporter involved in HIV1 entry into host cells. The structure consists of a trimer of two peptides, which form a transient hexameric helical bundle through the association of three C-terminal helices (C34) onto a central core formed by three N-terminal helices (N36). Highly conserved pockets on the N36 peptide core are potential therapeutic targets for mimetics of the complementary C34 peptide α-helical regions. The α-helix makes contacts through the *i*, *i* + 3, *i* + 4, and *i* + 7 residues of the helix, corresponding to Trp628, Trp631, Asp632, and Ile635.[25] Hamilton *et al.* developed mimetics of the hydrophobic face of the C34 α-helix using the terphenyl scaffold, which was successfully used to disrupt N36/C34 complexation.[26]

smMLCK complexed with CaM. Calmodulin (CaM) has a variety of functions in the cell cycle and interacts with a number of proteins, including smooth muscle myosin light chain kinase (smMLCK). It is theorized that $(Ca^{2+})_4$–CaM binds to smMLCK, activating it and inducing a signal cascade that leads to muscle contraction.[27] The crystal structure of CaM bound with the α-helical peptide of smMLCK shows that the binding occurs through the *i*, *i* + 4, and *i* + 7 residues of the

Table 1 Protein–protein interactions involving α-helices

Target	Helix	Diseases	Residues	Motif
Vav	N-terminus	Cancer	I173, Y174, L177	*i*, *i* + 1, *i* + 4
Tiam1	Rac1	Cancer	Q1191, K1195, L1198	*i*, *i* + 4, *i* + 7
ERα	GRIP1	Cancer	L690, L693, L694	*i*, *i* + 3, *i* + 4
TR	Tachykinin peptides	Neurological disorders		*i*, *i* + 1
NaIP	N-terminus	Bacterial infections	D792, D799, R803, K806, D810, D813	*i*, *i* + 4, *i* + 7
GP41	C34 peptide	HIV	W628, W631, D632, I635	*i*, *i* + 3, *i* + 4, *i* + 7
CaM	smMLCK	Cancer	W800, T803, V807	*i*, *i* + 4, *i* + 7
HDM2	P53	Cancer	F19, W23, L26	*i*, *i* + 4, *i* + 7
Bcl-xL	Bak	Cancer	V74, L78, I81, I85	*i*, *i* + 4, *i* + 7, *i* + 11

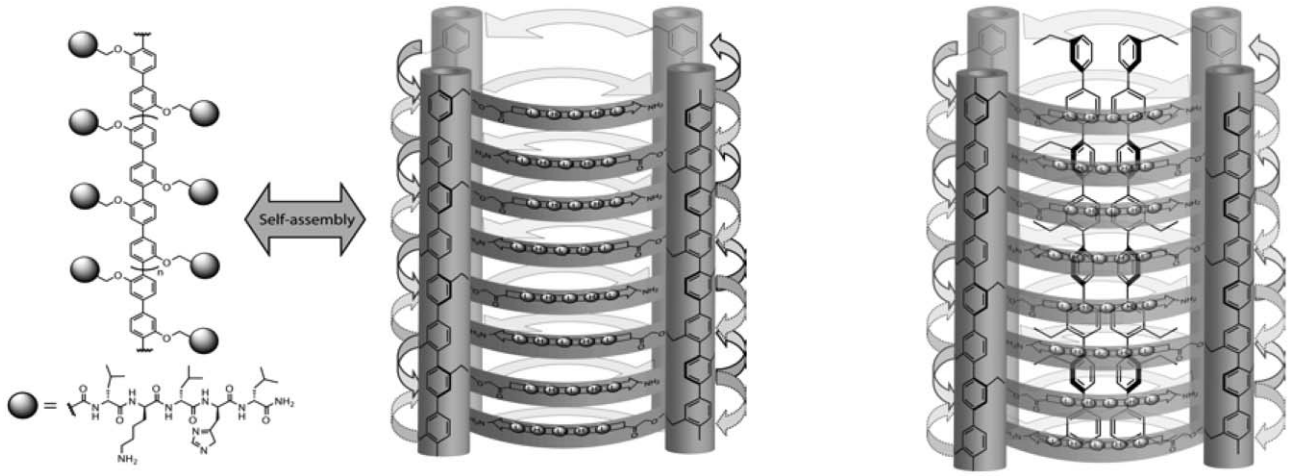

Fig. 4 (Left) Synthetic β-barrels described by Matile and co-workers. The four rigid-rods served as the "staves" of the barrel are obtained by the consecutive and extended octaphenyl as α-helix mimetics. Combined with intermolecular, interdigitating antiparallel β-sheets, Matile and co-workers developed structural and functional synthetic β-barrels. (Right) α-Helix mimetics blocking the synthetic barrel channel.

α-helix, corresponding to Trp800, Thr803, and Val807.[28] The terphenyl scaffold designed by Hamilton and co-workers was used to develop inhibitors of the CaM–smMLCK interaction. One derivative, showing an IC_{50} of 800 nM, is among the most potent CaM antagonists known.[29]

4.2 HDM2 complexed with p53

The over-expression of peptidic human double minute (HDM2), which is the human homolog of peptidic mouse double minute (MDM2), in cancer cells is known to inactivate p53, allowing for cell proliferation and prevention of apoptosis. Disruption of the HDM2–p53 complex has been shown to be a viable approach towards potential chemotherapeutics. The crystal structure of this complex reveals a hydrophobic binding pocket on HDM2 in which an α-helical region of p53 makes hydrophobic contacts through Phe19, Trp23, and Leu26, corresponding to the *i*, *i* + 4, and *i* + 7 residues of the helix.[30,31] Screening[32] and computational[33] techniques have been used to find inhibitors of this interaction. The Hamilton group has applied the terphenyl scaffold to target the HDM2–p53 interaction, finding an inhibitor with a K_i of about 180 nM.[1] Stoll *et al*. used rational design based on the antitumor activity of chalcones to develop inhibitors of the MDM2–p53 interaction (Fig. 5).[16] Cummings *et al.* used this interaction to develop thir BZD-based mimetics (Fig. 6).[18]

4.3 Bcl-2 Family

Bcl-2 family proteins regulate apoptosis and consist of 25 members that possess at least one Bcl-2 homology (BH3) domain. Some of these function as pro-survival proteins (including Bcl-2, Bcl-x_L, A1, Boo, Bcl-w, and Mcl-1) and others as pro-death proteins (including Bax, Bak, and Bok) members. All pro-death members contain the BH3 domain, which is essential for their function.[34–36]

The NMR solution structure of Bcl-x_L bound to the BH3 domain of Bak (Fig. 7) shows that residues Val74, Leu78, Ile81, and Ile85, corresponding to *i*, *i* + 4, *i* + 7 and *i* + 11, make hydrophobic contacts with the binding cleft of Bcl-x_L.[37] Screening techniques, *in vitro*[38] and *in silico*,[39] have been used to find inhibitors of the Bcl-2 family dimerization.

Hamilton and co-workers have developed inhibitors based on the terphenyl, terephthalamide, and oligoamide

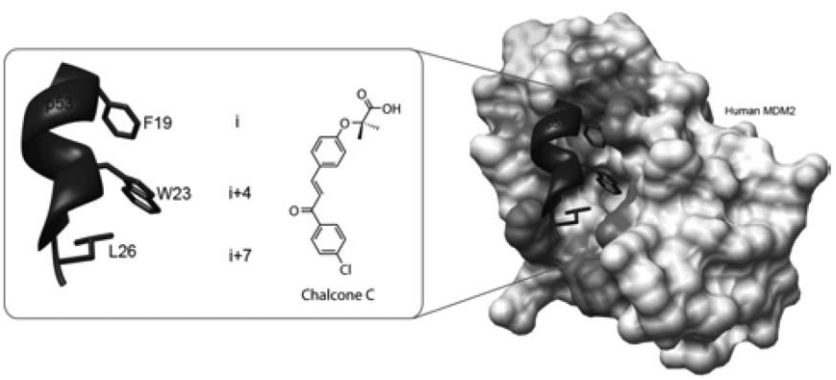

Fig. 5 X-ray crystal structure of the HDM2–p53 complex. The key side chains of p53: F19, W23, and L26 are shown in stick representation. The hydrophobic cleft of the binding interface of HDM2 is shaded. Stoll and co-workers showed that Chalcone derivatives adopted the extended conformation as they demonstrated a similar binding mode to that of p53.

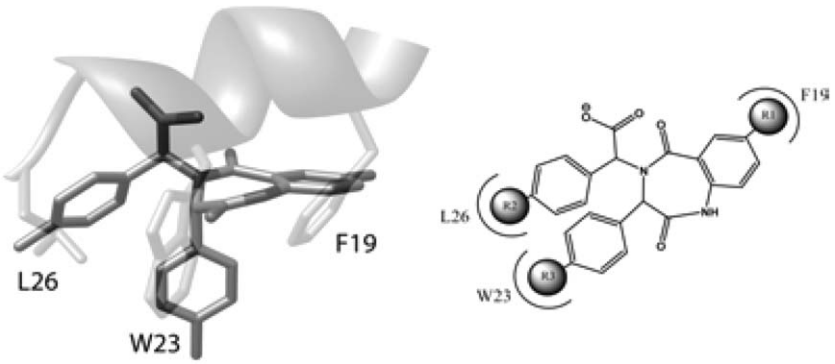

Fig. 6 BZD scaffold found by Cummings *et al.* as an α-helix mimetic.

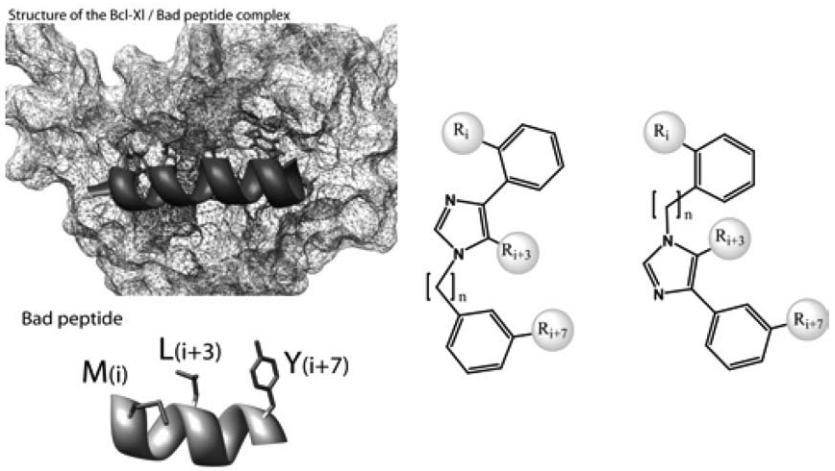

Fig. 7 Imidazole scaffold designed to inhibit the Bcl-x_L–Bad interaction.

foldamer scaffolds capable of disrupting the Bcl-x_L–Bak interaction.[14,40] Analysis of the imidazole scaffold developed by Antuch *et al.* using *in vitro* screening suggested that imidazole derivatives, conceptually derived from the Hamilton terphenyl scaffolds, mimic the *i*, *i* + 3 and *i* + 7 residues of Bad peptide (Fig. 7).[19]

4.4 Estrogen receptor–CoA complex

The estrogen receptor (ER) regulates the proliferation of various cell types and has been implicated in a number of diseases, including breast cancer. ER is regulated through agonists and antagonists that affect its structure. For example, binding to an antagonist such as 4-hydroxytamoxlate (OHT) induces a structural change that causes an inhibitory α-helical sequence of ER to dissociate and an α-helical region of GRIP1 to bind. In both cases, it is a LXXLL motif that binds to this hydrophobic groove, corresponding to the *i*, *i* + 3, *i* + 4 residues of the α-helices.[41] Because of importance of the ER in the search for therapeutics, a number of inhibitors have been developed based on the α-helical region of the CoA. Rodriguez *et al.* used a rational design approach to create small molecule LXXLL motif mimetics based on a tris-substituted triazine scaffold. Through this approach they found a co-activator binding inhibitor (CBI) with 30 μM affinity for ERα (Fig. 8).[20]

4.5 Tachykinin receptors

Tachykinin receptors (TR) are neuroreceptors that regulate neurological response by binding to tachykinin peptides. These peptides are about 10 amino acids long and form an α-helical secondary structure. The deregulation of TRs has been implicated in schizophrenia, Alzheimer's disease, and epilepsy.[42] The crystal structures for the tachykinin receptors are not available; however, based on the structure of the tachykinin peptides, TRs have been targets of α-helix mimetics.[43,44] Horwell *et al.* have found that 1,6-disubstituted indanes, designed to mimic mammalian tachykinin peptide residues, have micromolar affinity for the tachykinin target, tachykinin neuroreceptor NK_2.[9]

4.6 Nuclear receptors and Rho-GEFs: unexplored potential targets for synthetic mimetics

Nuclear receptors. The nuclear receptor (NR) family of proteins plays a regulatory role in transcription. The first subset of this family are steroid hormone binding NRs which are found in the cytoplasm in complex with chaperone proteins when not bound to ligand. Ligand binding induces the release of the chaperone proteins, leading to their dimerization, entrance into the nucleus, and binding to hormone response elements (HREs), thus leading to transcription. Non-steriod

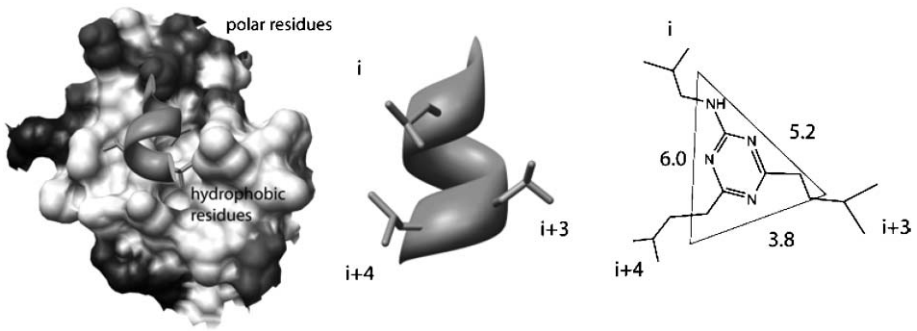

Fig. 8 The X-ray crystal structure of **GRIP1** peptide bound to estrogen receptor. The binding motif is shown as *i*, *i* + 3, *i* + 4 of the helix. The Class I of the Co-activator Binding Inhibitor (**CBI**) is shown with its relative positions and the distance between the ligands (unit: Å).

binding nuclear receptors are found in the nucleus bound as a complex that prohibits transcription. Ligand binding destabilizes this complex, allowing for transcription.[45]

Regulation of each type of nuclear receptor is achieved through co-regulatory proteins, or co-activators (CoAs), that bind through the activation function 2 (AF-2) domain located on the ligand binding domain (LBD) of the NR. The ligand binding domain consists of 12 α-helices. Upon ligand binding, the structure of the helices changes such that a new binding groove is created in which the CoA binds. This binding sequence ultimately leads to transcription.[45] Analysis of NR–CoA complexes reveals that the binding groove of the NR interacts with an α-helical region of the CoA containing a conserved LXXLL motif (Table 2).[46] These complexes are highly related, yet the estrogen receptor is the only published example that has been targeted through the nuclear receptor–CoA interaction.

Rho-GEFs. The Dbl family of proteins plays a key role in the regulation of cell growth and members of the family have been identified as oncogene products. These proteins are defined by a Dbl homology (DH) domain (named for diffuse B-cell lymphoma protein) which is N-terminal to a pleckstrin homology (PH) domain. Their role is as guanine nucleotide exchange factors (GEFs) for Rho family small GTP binding proteins (or G-proteins), catalyzing the release of GDP which permits the uptake of GTP. This function of the Dbl family is a part of the regulation of Rho signaling, where GTP Rho is active and capable of binding downstream targets and GDP bound Rho is inactive. The exchange of GDP for GTP is considered the rate limiting step of G-protein activity, therefore the catalytic role of the Dbl family is of great importance in cell growth regulation. Dbl-related GEF proteins include Dbl, Cdc24, Ost, Tiam-1, Ect-2, Vav, Lbc, FGD1, Dbs, Lfc, Tim, Brc, Abr, Sos, and Ras GEF. The DH domain of these proteins consists of a 3 helix core with a cylindrical packed structure. The GTPase activity lies outside this helical core and is exerted by binding to GDP-bound G-proteins, lowering their affinity for GDP and facilitating its release.[47] The PH domain is responsible for translocation of the GEF to the cell membrane, where mitogen signaling activates it. In some cases, it has been shown that the PH domain also serves a regulatory function.[48] Two possible mechanisms for control of GEF function through α-helix mimetics are discussed below: preventing the Dbl–Rho interaction and preventing Dbl autoinhibition.

Table 2 LXXLL motif (highlighted bold) of various CoAs belonging to the p160 family (HD = helical domain)

CoA	Sequence
SRC1, HD1	TSHK**LVQLL**TTT
SRC-1, HD2	RHKI**LHRLL**QEG
SRC-1, HD3	DHQL**LRYLL**DKD
P/CIP, HD1	GHKK**LLQLL**TCS
P/CIP, HD2	KHRI**LHKLL**QNG
P/CIP, HD3	NNAL**LRYLL**DRD
NCoA-2, HD1	GQTK**LLQLL**TTK
NCoA-2, HD2	KHKI**LHRLL**QDS
NCoA-2, HD3	ENAL**LRYLL**DKD
RXR-α	IDTF**LMEML**EAP
RAR-γ	MPPL**IREML**ENP

Tiam1 in complex with Rac1. Tiam1 is a Dbl protein that is involved in T-lymphoma invasion and metastasis. It is found in almost all analyzed tumor cell lines. Worthylake *et al.* solved the crystal structure of Tiam1 in complex with Rac1, revealing an interaction between the Tiam1 DH domain and the switch regions of Rac1.[49] These interactions change the structure of the nucleotide-binding cleft of Rac1, causing the release of GDP.[50]

The Tiam1–Rac1 interaction reveals an α-helix in the switch 2 region of Rac1 making contacts with Tiam1 through Gln1191, Lys1195, and Leu1198, corresponding to the *i*, *i* + 4, and *i* + 7 residues of the α-helix. Mimicking this α-helix may block the protein–protein interaction, thereby preventing GDP–GTP exchange and Tiam1 activation.

Although there are no published inhibitors of the Tiam1–Rac1 interaction based on α-helical mimicry, this approach is validated through work of Gao *et al.* Through structure based virtual screening, Gao *et al.* found a compound that can inhibit Rac1–GEF interactions.[51]

Vav autoinhibition. Vav is a Dbl protein involved in signaling pathways that control proliferation, cytoskeletal growth, and apoptosis in lymphoid cells. Based on the NMR solution structure combined with previous *in vitro* and *in vivo*

studies, Rosen *et al*. proposed a possible mechanism for Vav autoinhibition and activation involving an *i*, *i* + 1, and *i* + 4 α-helical motif. Mechanistic work has shown that Vav autoinhibition is relieved by Src-family kinases, activating the catalytic function of the DH domain of Vav.[52]

The NMR solution structure of Vav reveals an N-terminal acidic region that forms an α-helix and binds across the phosphorylation active site of the DH domain. This α-helix contains an IYXXL motif (where X is an acidic residue) corresponding to Ile173, Tyr174, and Leu177 (Table 1). Phosphorylation of Tyr174 is a key step in relieving the autoinhibition of Vav and the solution structure shows this residue to be flanked by hydrophobic contacts formed by Ile173 and Leu177 in the binding cleft of the DH domain. By reproducing these key residues within a small molecule, it may be possible to block the active site, mimicking the autoinhibition mechanism of Vav and preventing its activity as a GEF.

5 Gaining selectivity through mimetic design

One question that remains to be answered is: how do we gain selectivity when mimicking a conserved motif among a family of proteins? One way to answer this question is to investigate how nature accomplishes this task. There are often subtle differences in the binding clefts that can be exploited through various functional groups in the inhibitor design. This type of analysis can be informed through computational investigation[53] and refined through SAR. For example, the terphenyl scaffold can show selectivity between HDM2 and Bcl-x_L *via* placement of a naphthyl substituent.

In creating a true mimetic, we may also exploit the differences in non-conserved regions of the α-helix. For example, the differences in the peptide chain between SRC1 and RAR show the SRC1 LXXLL motif flanked by a terminal amine and phenol, while the RAR LXXLL motif is flanked by a hydrophobic residue and a negatively charged glutamic acid (Table 2).[46] Mimicking these differences may be the answer to selective inhibitor design.

Conclusions

The design of small molecule mimetics of large protein surfaces represents a growing field in medicinal chemistry. The plethora of potential targets that have been identified over the past 20 years through the application of NMR and crystallographic techniques has allowed the use of structure information in the rational design of such mimetics. One frequently used motif in biological interactions is the α-helix. Various groups have exploited this in designing inhibitors of protein–protein and protein–DNA interactions based on an α-helical mimetic approach. Still, this tactic remains in its infancy, with many potential targets left unexplored.

Acknowledgements

We thank the National Institute of Health (GM 69850) for financial support of the work described in this review that was carried out at Yale.

References

1 H. Yin, G. I. Lee, H. S. Park, G. A. Payne, J. M. Rodriguez, S. M. Sebti and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2005, **44**, 2704.
2 U. Lehnert, Y. Xia, T. E. Royce, C. S. Goh, Y. Liu, A. Senes, H. Y. Yu, Z. L. Zhang, D. M. Engelman and M. Gerstein, *Q. Rev. Biophys.*, 2004, **37**, 121.
3 D. J. Barlow and J. M. Thornton, *J. Mol. Biol.*, 1988, **201**, 601.
4 R. Schwartz, S. Istrail and J. King, *Protein Sci.*, 2001, **10**, 1023.
5 S. Kumar and M. Bansal, *Proteins: Struct., Funct., Bioinf.*, 1998, **31**, 460.
6 A. R. Curran and D. M. Engelman, *Curr. Opin. Struct. Biol.*, 2003, **13**, 412.
7 D. C. Horwell, W. Howson, W. P. Nolan, G. S. Ratcliffe, D. C. Rees and H. M. G. Willems, *Tetrahedron*, 1995, **51**, 203.
8 D. C. Horwell, W. Howson, G. Ratcliffe and H. Willems, *Bioorganic & Medicinal Chemistry Letters*, 1994, **4**, 2825.
9 D. C. Horwell, W. Howson, G. S. Ratcliffe and H. M. G. Willems, *Bioorg. Med. Chem.*, 1996, **4**, 33.
10 O. Kutzki, H. S. Park, J. T. Ernst, B. P. Orner, H. Yin and A. D. Hamilton, *J. Am. Chem. Soc.*, 2002, **124**, 11838.
11 H. Yin and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2005, **44**, 4130.
12 J. M. Davis, A. Truong and A. D. Hamilton, *Org. Lett.*, 2005, **7**, 5405.
13 I. C. Kim and A. D. Hamilton, *Org. Lett.*, 2006, **8**, 1751.
14 H. Yin, G. I. Lee, K. A. Sedey, J. M. Rodriguez, H. G. Wang, S. M. Sebti and A. D. Hamilton, *J. Am. Chem. Soc.*, 2005, **127**, 5463.
15 S. Litvinchuk and S. Matile, *Supramol. Chem.*, 2005, **17**, 135.
16 R. Stoll, C. Renner, S. Hansen, S. Palme, C. Klein, A. Belling, W. Zeslawski, M. Kamionka, T. Rehm, P. Muhlhahn, R. Schumacher, F. Hesse, B. Kaluza, W. Voelter, R. A. Engh and T. A. Holak, *Biochemistry*, 2001, **40**, 336.
17 H. Oguri, A. Oomura, S. Tanabe and M. Hirama, *Tetrahedron Lett.*, 2005, **46**, 2179.
18 M. D. Cummings, C. Schubert, D. J. Parks, R. R. Calvo, L. V. LaFrance, J. Lattanze, K. L. Milkiewicz and T. B. Lu, *Chem. Biol. Drug Design*, 2006, **67**, 201.
19 W. Antuch, S. Menon, Q. Z. Chen, Y. C. Lu, S. Sakamuri, B. Beck, V. Schauer-Vukasinovic, S. Agarwal, S. Hess and A. Domling, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 1740.
20 A. L. T. Rodriguez, A. Tamrazi, M. L. Collins and J. A. Katzenellenbogen, *J. Med. Chem.*, 2004, **47**, 600.
21 A. G. Cochran, *Curr. Opin. Chem. Biol.*, 2001, **5**, 654.
22 M. Arkin, *Curr. Opin. Chem. Biol.*, 2005, **9**, 317.
23 C. J. Oomen, P. van Ulsen, P. Van Gelder, M. Feijen, J. Tommassen and P. Gros, *EMBO J.*, 2004, **23**, 1257.
24 J. Y. Winum and S. Matile, *J. Am. Chem. Soc.*, 1999, **121**, 7961.
25 D. C. Chan, D. Fass, J. M. Berger and P. S. Kim, *Cell*, 1997, **89**, 263.
26 J. T. Ernst, O. Kutzki, A. K. Debnath, S. Jiang, H. Lu and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2001, **41**, 278.
27 J. E. Van Lierop, D. P. Wilson, J. P. Davis, S. Tikunova, C. Sutherland, M. P. Walsh and J. D. Johnson, *J. Biol. Chem.*, 2002, **277**, 6550.
28 W. E. Meador, A. R. Means and F. A. Quiocho, *Science*, 1992, **257**, 1251.
29 B. P. Orner, J. T. Ernst and A. D. Hamilton, *J. Am. Chem. Soc.*, 2001, **123**, 5382.
30 P. H. Kussie, S. Gorina, V. Marechal, B. Elenbaas, J. Moreau, A. J. Levine and N. P. Pavletich, *Science*, 1996, **274**, 948.
31 J. P. Blaydes and D. Wynford-Thomas, *Oncogene*, 1998, **16**, 3317.
32 B. L. Grasberger, T. B. Lu, C. Schubert, D. J. Parks, T. E. Carver, H. K. Koblish, M. D. Cummings, L. V. LaFrance, K. L. Milkiewicz, R. R. Calvo, D. Maguire, J. Lattanze, C. F. Franks, S. Y. Zhao, K. Ramachandren, G. R. Bylebyl, M. Zhang, C. L. Manthey, E. C. Petrella, M. W. Pantoliano, I. C. Deckman, J. C. Spurlino, A. C. Maroney, B. E. Tomczuk, C. J. Molloy and R. F. Bone, *J. Med. Chem.*, 2005, **48**, 909.
33 P. S. Galatin and D. J. Abraham, *J. Med. Chem.*, 2004, **47**, 4163.
34 J. M. Adams and S. Cory, *Trends Biochem. Sci.*, 2001, **26**, 61.
35 J. C. Reed, *Nature*, 1997, **387**, 773.

36 A. Strasser, D. C. S. Huang and D. L. Vaux, *Biochim. Biophys. Acta: Rev. Cancer online*, 1997, **1333**, F151.
37 M. Sattler, H. Liang, D. Nettesheim, R. P. Meadows, J. E. Harlan, M. Eberstadt, H. S. Yoon, S. B. Shuker, B. S. Chang, A. J. Minn, C. B. Thompson and S. W. Fesik, *Science*, 1997, **275**, 983.
38 A. Degterev, A. Lugovskoy, M. Cardone, B. Mulley, G. Wagner, T. Mitchison and J. Y. Yuan, *Nat. Cell Biol.*, 2001, **3**, 173.
39 I. J. Enyedy, Y. Ling, K. Nacro, Y. Tomita, X. H. Wu, Y. Y. Cao, R. B. Guo, B. H. Li, X. F. Zhu, Y. Huang, Y. Q. Long, P. P. Roller, D. J. Yang and S. M. Wang, *J. Med. Chem.*, 2001, **44**, 4313.
40 H. Yin, G. I. Lee, K. A. Sedey, O. Kutzki, H. S. Park, B. P. Omer, J. T. Ernst, H. G. Wang, S. M. Sebti and A. D. Hamilton, *J. Am. Chem. Soc.*, 2005, **127**, 10191.
41 A. K. Shiau, D. Barstad, P. M. Loria, L. Cheng, P. J. Kushner, D. A. Agard and G. L. Greene, *Cell*, 1998, **95**, 927.
42 A. K. Mantha, I. R. Chandrashekar, N. Z. Baquer and S. M. Cowsik, *J. Biomol. Struct. Dyn.*, 2004, **22**, 137.
43 J. N. Pennefather, A. Lecci, M. L. Candenas, E. Patak, F. M. Pinto and C. A. Maggi, *Life Sci.*, 2004, **74**, 1445.
44 T. A. Almeida, J. Rojo, P. M. Nieto, F. M. Pinto, M. Hernandez, J. D. Martin and M. L. Candenas, *Curr. Med. Chem.*, 2004, **11**, 2045.
45 C. Rochette-Egly, *J. Biol. Chem.*, 2005, **280**, 32565.
46 M. J. Plevin, M. M. Mills and M. Ikura, *Trends Biochem. Sci.*, 2005, **30**, 66.
47 G. R. Hoffman and R. A. Cerione, *FEBS Lett.*, 2002, **513**, 85.
48 B. Aghazadeh, W. E. Lowry, X.-Y. Huang and M. K. Rosen, *Cell*, 2000, **102**, 625.
49 K. L. Rossman, D. K. Worthylake, J. T. Snyder, D. P. Siderovski, S. L. Campbell and J. Sondek, *EMBO J.*, 2002, **21**, 1315.
50 D. K. Worthylake, K. L. Rossman and J. Sondek, *Nature*, 2000, **408**, 682.
51 Y. Gao, J. B. Dickerson, F. Guo, J. Zheng and Y. Zheng, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 7618.
52 G. K. Amarasinghe and M. K. Rosen, *Biochemistry*, 2005, **44**, 15257.
53 M. Pastor and G. Cruciani, *J. Med. Chem.*, 1995, **38**, 4637.

