# Utilizing Peptide Structures As Keys For Unlocking Challenging Targets

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**Abstract:** Three-dimensional structures of protein targets have proven to be extremely valuable for modern drug design and discovery. For cases where the structure of the protein is unattainable, such as G-protein coupled receptors (GPCRs), structural information on active ligands is still useful and helpful for deciphering the geometrical and chemical features of the active site. Peptides, constructed from easy-to-form amide backbones and featuring variable side-chains, have an inherent advantage in generating rapid quantitative structure-activity relationships (QSAR). Given the fact that peptides are natural ligands for many protein targets, structural investigation of a series of related peptides, typically carried out *via* nuclear magnetic resonance (NMR), can result in an accurate pharmacophore model. Such a model can be used for virtual screening, and to assist design of second-generation peptidomimetics with improved properties and design of non-peptidic leads. In this article, we will review examples in which a structural approach utilizing peptide ligands was employed to obtain a better understanding of the target active site. We will focus on cases where such information supplied guidance toward the discovery of small molecule ligands.

**Key Words:** Structure of peptide, NMR, pharmacophore, small molecule drug design.

## INTRODUCTION

Over the past two decades, peptide research in drug discovery has been viewed as a capability of declining value. This situation is primarily caused by the increasing emphasis on one of the most important concepts in the pharmaceutical industry – druglikeness [1]. Focus has been placed on the ability to efficiently identify and reject compounds that lack the necessary properties to become a drug, since a high attrition rate of drug candidates at the expensive clinical trial stage has long been an issue affecting the overall efficacy of the pharmaceutical industry [2]. Detailed analysis has indicated that the failure of drug candidates is largely attributable to their poor absorption, distribution, metabolism, elimination and toxicology (ADMET) properties [3]. The approach of frontloading ADMET considerations into the pre-clinical stage gave birth to the practice of examining compounds for druglikeness and setting up rejection criterion based on these properties [1,4-6]. Peptides suffered under this approach, because they are not viewed as druglike, due to their short biological half-lives and poor oral availability. This view may be shifting recently due to the development of novel drug delivery systems [7,8]. Nevertheless, the capacity to produce and study peptides has been downgraded in most drug discovery organizations. This is unfortunate, because structural information extracted from peptides may provide a shortcut to the discovery of potent small molecule drugs. Further, peptides offer the opportunity for rapidly addressing another important yet problematic concept in drug discovery – chemical diversity [9,10].

Peptides are the natural substrates of many important protein classes, such as proteases, peptidases, polymerases, and G-protein coupled receptors (GPCRs), so it is often possible for a drug discovery program to start with a known peptide substrate. In addition, protein-protein interactions can sometimes be studied by simplifying one of the participants into the form of an active peptide fragment.

Peptides are biopolymers constructed from easy-to-form amide bonds and the building blocks, typically $\alpha$-amino acids, are commercially available, offering a large selection of functional side-chains. Consequently, it is relatively easy to generate a large number of rationally varied test compounds, and thereby rapidly derive quantitative structure-activity relationships (QSAR). Further, certain peptides exhibit a defined structure, which can be determined experimentally by NMR, so the activities can be correlated with three-dimensional spatial distributions of key functional groups.

The goal of this review is to call attention to the important roles peptides can play in pharmaceutical research by describing selected stories that started with peptides, and outlining the strategies of utilizing peptides as keys to unlock important targets. In each case selected, enough structural information has become available to understand in detail how the small molecule drug compounds ultimately were able to mimic the original peptide ligands.

### BCL-XL

BCL-XL, a member of the BCL-2 protein family, plays a key role in programmed cell death, or apoptosis [11]. The BCL-2 protein family is comprised of both anti-apoptosis proteins, such as BCL-2 and BCL-XL, and pro-apoptosis proteins, such as Bak and Bad. Apoptosis is regulated by heterodimerization between members of BCL-2 family. All the pro-apoptosis proteins possess the BCL-2 homology 3 (BH3) domain. The interaction between BCL-XL and the BH3 region of another family member can inhibit cell survival and induce apoptosis. This regulatory function of BCL-XL in apoptosis makes it an important target for oncology [12].

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The solution structure of BCL-XL and a 16-residue peptide derived from BH3 region of Bak was determined by Fesik and coworkers [13]. The 16-residue peptide bound to BCL-XL tightly with a $K_D$ of 0.34 μM, while a truncated 11-residue peptide demonstrated no activity. The 16-residue Bak peptide adopted an amphipathic α–helical structure, which interacted with BCL-XL by projecting four hydrophobic side chains on one side of the peptide, $Val^{74}$, $Leu^{78}$, $Ile^{81}$, and $Ile^{85}$, into a hydrophobic cleft formed by α–helices of BCL-XL. A 25-residue peptide comprising the BH3 region of Bad, another downstream partner, bound to BCL-XL more tightly, with a $K_D$ of 0.6 nM. This increased affinity was ascribed to an increased helix propensity of the Bad peptide [14]. The NMR structure of the BCL-XL/Bad complex demonstrated a similar binding mode for Bad, with the side chains of three hydrophobic residues, $Tyr^{147}$, $Leu^{151}$, and $Phe^{158}$, completely buried in the ligand-binding cleft of BCL-XL [14] (Fig. **1a**).

The ligand binding site of BCL-XL is relatively large and somewhat exposed to the solvent, making it a challenge to design small molecule inhibitors for BCL-XL. To date, the most potent BCL-XL non-peptide inhibitor reported has been a molecule of molecular weight 815, discovered at Abbott [15]. ABT-737 binds tightly to both BCL-XL and BCL-2 with $K_i$ < 1nM, and it also exhibits high efficacy against three different chromosomal translocation-containing lymphoma cell lines RS11380, DoHH2 and SuDHL-4 with $EC_{50}$ values of 0.15 μM, 0.13 μM, and 0.85 μM respectively [15]. It was not designed directly from a peptide, but developed following identification of small molecules that bound to the active site, using an NMR screening approach [15]. The amount of optimization was substantial, as the final compound looks very different from the initial small molecule hits. Presumably this optimization was guided by structural knowledge of how peptide ligands bind to BCL-XL and BCL-2. ABT-737 was shown to bind to the same hydrophobic groove to which Bak and Bad peptides bind. A comparison of the bound structures of ABT-737 and the Bad peptide is shown in Fig. **1b**. ABT-737 adopts a C-shape with a phenyl ring at one end, and two stacked phenyl rings at the other, projecting out to form hydrophobic interactions with the BCL-XL pocket. Its long edge superimposes well with the backbone of the Bad peptide. The three key hydrophobic side chains of the Bad peptide do not all superimpose exactly onto moieties of ABT-737. However, $Phe^{158}$ is directly mimicked by the folded back terminal phenyl ring of ABT-737, and $Leu^{151}$ is mimicked to some extent by the chloro-biphenyl group at the other end of ABT-737. The successful

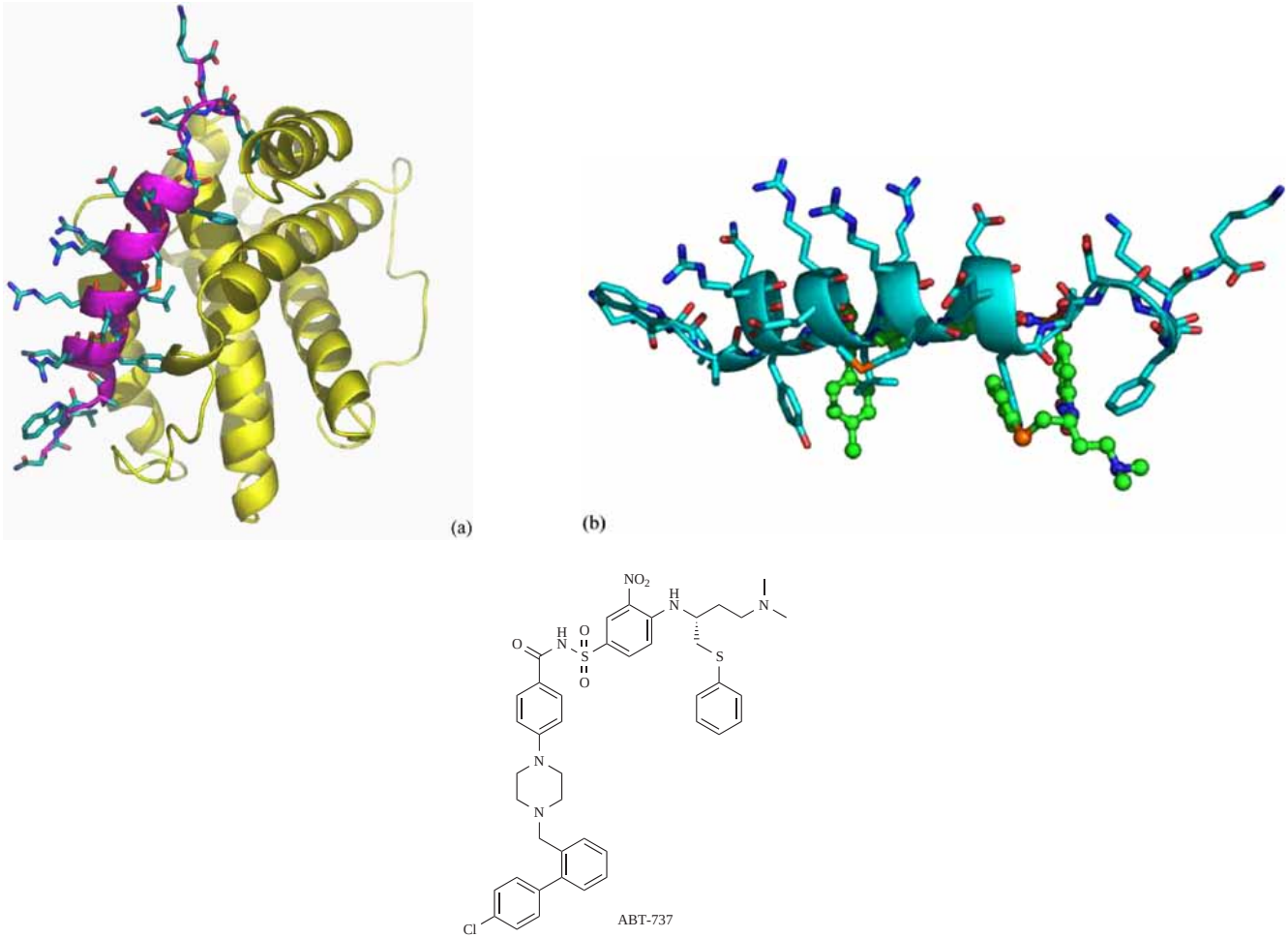



**Fig. (1).** (a) Solution structure of BCL-XL (colored in yellow) in complex with Bad peptide (colored in magenta); (b) Superposition of 25-residue Bad peptide (colored in cyan) and ABT-737 (colored in green). Figure was generated with PyMOL (Delano Scientific).

development of ABT-737 demonstrated that a small molecule can ultimately achieve equivalent or better binding affinity than a peptide counterpart, and that in doing so, replicates some but not all of the interactions the peptide makes with the protein.

In a separate attempt to discover novel small molecule inhibitors of BCL-XL, the Hamilton lab at Yale University used a direct design approach. They designed a series of helical structure mimetics based on terphenyl and terephthalamide scaffolds [16,17]. Both scaffolds were found to be capable of projecting three vectors in the same direction as the side chains of residues *i*, *i + 4* and *i + 7* in an α-helix. (Fig. **2**) This strategy was used to mimic the interactions of peptide ligands of BCL-XL. The most potent terphenyl compound, which projected isobutyl, naphthalene, and isobutyl groups, exhibited a $K_i$ value of 0.114 μM and disruption of the binding of BCL-XL to Bax was observed after treating human embryonic kidney 293 (HEK293) cells with the compound at a concentration of 100μM [17]. The terephthalamides, which exhibited improved physicochemical properties, showed an *in vitro* potency of 0.78 μM, and an improved cell based activity of $IC_{50}$ 35.0 μM [16,18]. Although the activities of these protein secondary structure mimetics are not yet near levels that would have therapeutic possibilities, and their ADMET properties are not optimal, the approach is promising, and again demonstrates that mimicking a peptide is an efficient strategy for discovery of a small non-peptidic ligand.

**MC4R**

The melanocortins are a family of peptides comprised of the α-, β-, and γ-melanocyte stimulating hormones (MSH) and adreno-corticotropic hormone. They have been implicated in numerous biological functions, including regulation of skin pigmentation, regulation of steroid production, modulation of the immune response, thermoregulation, obesity, and sexual function. The physiological effects of these peptides are mediated through five G-protein-coupled receptor subtypes, designated MC1-5R. MC4R is of particular interest for drug discovery because it influences food intake and modulates erectile activity. Therefore, an agonist of MC4R might find use as a treatment for obesity or sexual dysfunction.

α-MSH is a thirteen-residue peptide, but the minimal active sequence was found to involve a core consisting of only four residues - $His^6$-$Phe^7$-$Arg^8$-$Trp^9$ [19] (Table **1**). Substitution at position 7 with (D)Phe yielded an increase in potency [20], and this was hypothesized to result from stabilization of a β-turn conformation. The structure of α-MSH containing the (D)Phe substitution was examined by NMR [21], and although this peptide was not overly rigid, a preference for a hairpin turn was reported. A breakthrough in understanding the active conformation came *via* the development of a cyclic peptide with exceptional potency - Ac-Nle-c[Asp-His-(D)Phe-Arg-Trp-Lys]-$NH_2$, which was designated "MT-II" [22]. This cyclic constraint allowed the conformation of the backbone to be much better defined, and NMR studies indicated a β-turn encompassing the region $Asp^5$-$His^6$-(D)$Phe^7$-$Arg^8$ [23]. The conformation of a version of the MT-II peptide, with proline substituted for $His^6$, was studied *via* NMR by researchers at Amgen, and this peptide was also found to adopt a turn-like conformation [24]. A further exploration of substitutions using unnatural amino acids was carried out at Roche, ultimately leading to an extremely constrained, highly potent analog - penta-c[Asp-Apc-(D)Phe-Arg-(2S,3S)-β-methylTrp-Lys]-$NH_2$, where Apc designates 1-amino-4-phenylcyclohexane-carboxylic acid. NMR studies showed that the backbone of this peptide exhibited a β-turn encompassing Asp-Apc-(D)Phe-Arg, (Fig. **3a**) and that even the side-chains were being held in specific conformations [23].

A drug development program searching for agonists of a system such as MC4R depends critically on the structure of peptide ligands because, being a membrane-bound GPCR, an experimental structure of the receptor will not be obtainable. The first such attempts at utilizing structural information for

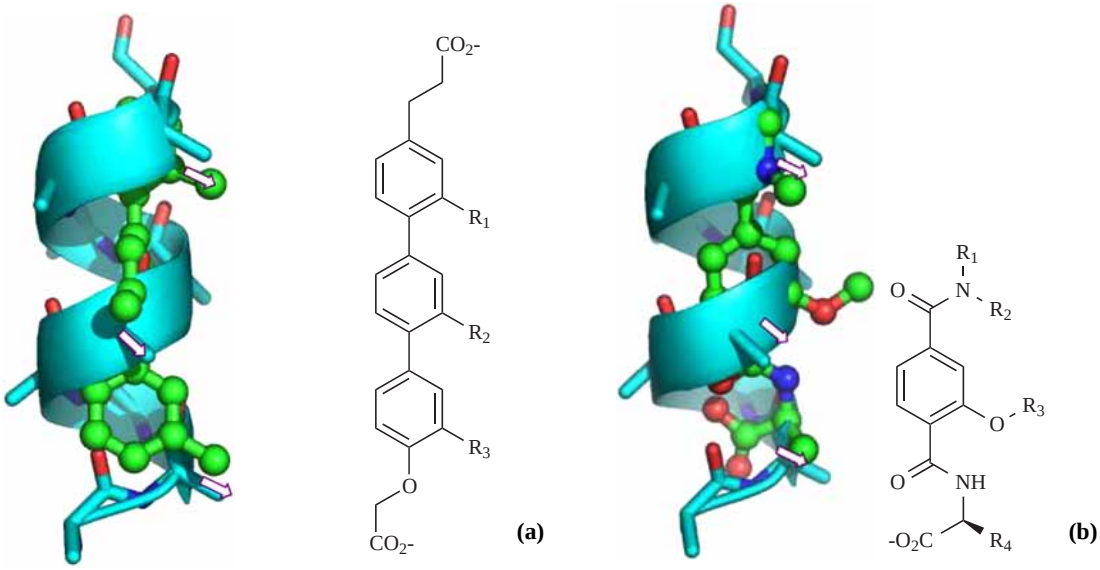



**Fig. (2).** Superposition of poly-Ala with (a) terphenyl α–helical mimetics and (b) terephthalamide α–helical mimetics. Arrows indicate projection of side chains. Figure was generated with PyMOL (Delano Scientific).

**Table 1. Amino Acid Sequence and MC4R Efficacy of Melanocortin Peptide Agonists**

| Peptide | Sequence | $EC_{50}$ (MC4R) |
|---|---|---|
| α-MSH | Ac-Ser[1]-Tyr[2]-Ser[3]-Met[4]-Glu[5]-**His[6]-Phe[7]-Arg[8]-Trp[9]**-Gly[10]-Lys[11]-Pro[12]-Val[13]-$NH_2$ | 2.1 nM |
| MT-II | Ac-Nle-cyclo(D-K)-Asp-**His[6]-(D)Phe[7]-Arg[8]-Trp[9]**-Lys-$NH_2$ | 0.6 nM |
| Roche | Penta-cyclo(D-K)-Asp-**Apc[6]-(D)Phe[7]-Arg[8]-*(2S,3S)* - *β-methyl*Trp[9]**-Lys-$NH_2$ | 11.0 nM |

the MC-4 system were based on the non-detailed assumption that the bioactive conformation of the natural peptide ligand was a β-turn. A library of compounds designed to mimic a generic β-turn were synthesized and screened for agonist activity against the MC-1 receptor [25]. Two compounds were identified, exhibiting micromolar levels of potency, and these represented the first non-peptidic agonists discovered for this system. Later, researchers at Amgen used their more detailed NMR structure of the cyclic peptide MT-II derivative for compound design. Using a cyclohexane core scaffold, compounds were designed that exhibited low nM potency as agonists of the MC-4 receptor [24]. This was a success in terms of direct design of peptidomimetics, although the compounds did not have desirable pharmacokinetic properties. Researchers at Merck were also guided by the conformational properites of a derviative of MT-II in their search for an MC4R agonist. A directed search of their compound collection was performed in an attempt to find a good mimic of the His-(D)Phe-Arg-Trp core pharmacophore. Initial hits were compounds of the spiroindanyl piperidine class. Following optimization, substituted piperidines with sub-nanomolar actvity towards MC4R were achieved, and they showed exquisite selectivity with regard to the other melanocortin receptors [26].

It is instructive to compare this most potent class of small molecule agonists with the most rigid active peptide. A superposition of the lowest energy conformation of one of the piperidine agonists upon the structure of the Apc-containing cyclic peptide [23] shows that the (D)Phe, Apc,

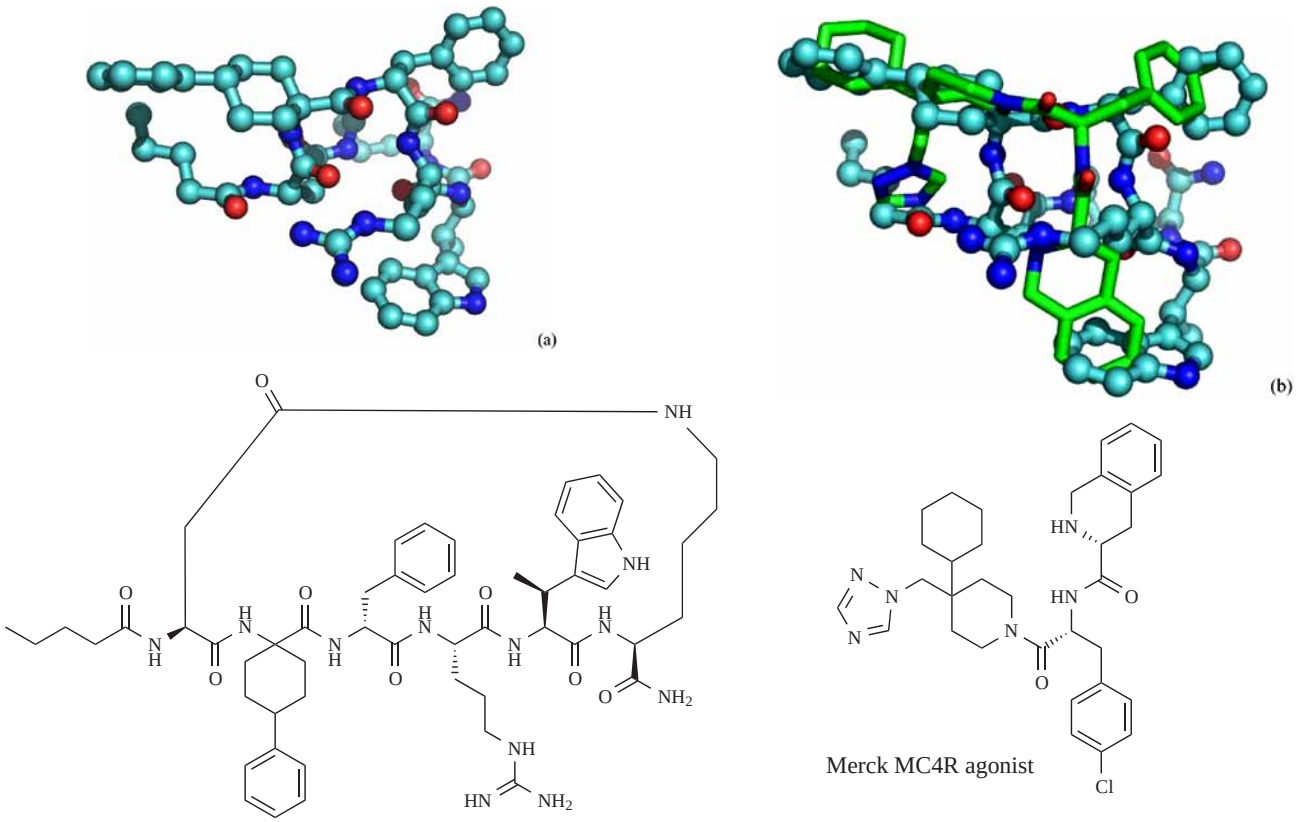



**Fig. (3).** (a) NMR structure of rigid MC4R agonist, cyclic peptide penta-c[Asp-Apc-(D)Phe-Arg-(2S,3S)-β-methylTrp-Lys]-$NH_2$; (b) Hypothetic superposition of peptide penta-c[Asp-Apc-(D)Phe-Arg-(2S,3S)-β-methylTrp-Lys]-$NH_2$ and a nonpeptide MC4R agonist synthesized by Merck. Figure was generated with PyMOL (Delano Scientific).

and combined Trp and Arg sites are effectively mimicked by a chlorophenylalnine, a cyclohexane, and a tetrahydro-isoquinoline group, respectively. (Fig. **3b**) This hypothetical superposition was recently supported experimentally by a study in which mutagenesis was applied to establish docking modes between the MC4 receptor and its peptide and nonpeptide agonists [27]. Given this encouraging agreement, the cyclic peptide should be effective as a tool for discovery of further scaffolds. To test this approach, a virtual screen was run using a pharmacophore derived from the peptide, and it was able to successfully select potent ligands that had been seeded into a large library of random drug-like molecules [23].

**MDM2**

The function of MDM2 is to regulate the level and activity of a tumor suppressor known as p53. It accomplishes this by binding to p53 and performing two actions: sterically blocking the transcription activation domain, and mediating the attachment of ubiquitin which marks p53 for subsequent elimination by the proteosome. MDM2 is overexpressed in certain human tumors. Restoring p53 function by inhibiting its interaction with MDM2 is viewed as a possible anti-cancer strategy.

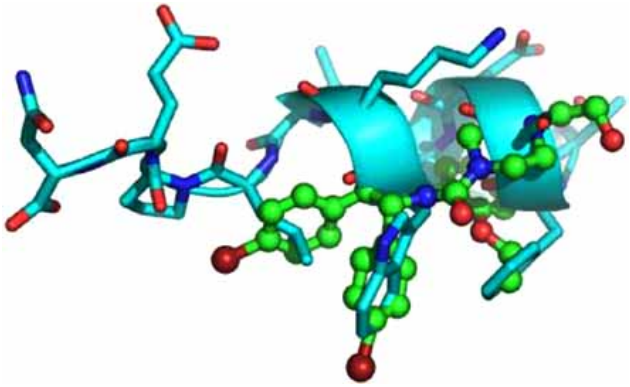
The portion of p53 that interacts with MDM2 was mapped to its N-terminal region, and ultimately short peptide fragments of p53 were identified that exhibited binding affinites for MDM2 comparable to the parent protein [28]. An X-ray structure was reported for the p53-binding domain of MDM2 complexed with such a peptide, comprised of residues 15-29 of p53 ($Ser^{15}$-$Gln^{16}$-$Glu^{17}$-$Thr^{18}$-$Phe^{19}$-$Ser^{20}$-$Asp^{21}$-$Leu^{22}$-$Trp^{23}$-$Lys^{24}$-$Leu^{25}$-$Leu^{26}$-$Pro^{27}$-$Glu^{28}$-$Asn^{29}$) [29]. This structure showed that the peptide adopts an α-helical conformation when bound, and achieves affinity by inserting three hydrophobic side chains - $Phe^{19}$, $Trp^{23}$, and $Leu^{26}$ - into sub-pockets within a binding cleft on MDM2. Free in aqueous solution, the peptide has no stable structure [30].

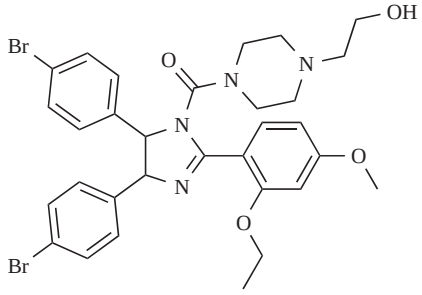
These p53 peptides were modified for optimal binding potency, by using the X-ray structure of the complexed peptide for guidance. Ultimately, a peptide analog was developed with a 1700-fold improvement in affinity (to 5nM), which was comprised of p53 residues 19-26 wherein $Asp^{21}$, $Leu^{22}$, $Trp^{23}$, and $Leu^{25}$ had been replaced with α-aminobutyric acid, phosphonomethyl-Phe, 6-chloro-Trp, and 1-amino-cyclopropanecarboxylic acid respectively [31]. Other analogs, much more different chemically from the original, were also developed using the helical structure of the bound peptide as a template. These included retro-inverso analogs [32] (affinity to 15 μM) and β peptides [33] (affinity to 233 nM). Also, cyclic peptides (affinity to 140 nM) were developed that, although designed to form a beta hairpin backbone, were able to project the key $Phe^{19}$, $Trp^{23}$, and $Leu^{26}$ side chains into the proper locations [34].

The MDM2-bound structure of the p53 peptide was utilized to assist discovery of small non-peptidic inhibitors as well. In one case, a pharmacophore derived from the peptide served as a template for virtual screening of the NCI chemical database, resulting in identification of a sulfonamide compound with 32 μM affinity [35]. In other cases, small molecules were designed de novo, directly from the peptide. In one study, a library of designed 2-phenoxy-benzoyl-tryptophan derivatives was synthesized, yielding a 100 nM inhibitor [36]. The Hamilton lab utilized their terphenyl scaffold, already proven to be a generic α-helical mimic, to develop a p53-MDM2 inhibitor with 1μM potency [37]. Another structure-based design and optimization effort produced a compound with a spiro-oxindole-3,3'-pyrrolidine scaffold that exhibited 86 nM affinity *in vitro*, and showed inhibition of tumor cell growth [38].

Other small molecule inhbitors of the MDM2-p53 interaction were discovered by a different approach, high-throughput screening using a diverse library, that did not rely immediately on the structures of p53 peptides. At 3D Pharmaceuticals (now Johnson and Johnson), benzodiazepine-carboxylic acids were identified from such a screen. Optimization benefited from X-ray structures of MDM2 complexed with small molecules from this class and with certain potent peptides, and ultimately produced compounds that exhibited < 100nM affinity and were able to decrease proliferation of tumor cells *in vivo* [39].

At Roche, small molecules have been reported that show very high potency and extremely promising *in vivo* activity. The published MDM2 structure complexed with the p53 peptide served the purpose of verifying that the binding pocket had sufficient dimensions to support tight binding of a drug-like small molecule, making a drug discovery






Nutlin-2

**Fig. (4).** Superposition of p53 peptide (colored by cyan) and Nutlin-2 (colored in green). Figure was generated with PyMOL (Delano Scientific).

program viable [40]. Screening identified a number of compounds that appeared to be active antagonists of the p53-MDM2 interaction. One such class of compounds was a group of imidazolines, which were subsequently optimized for potency, selectivity, and drug-like physical properties. Ultimately, compounds were developed (the "nutlins") that exhibited *in vitro* binding potencies as low as 90 nM, and showed growth inhibition of human tumor xenografts in mice [41]. High-resolution structures of some of the nutlins bound to MDM2 [41,42] allow evaluation of the strategy by which these drug-like molecules replicate the natural protein ligand. An overlay of the bound structure of the imidazoline with that of the bound p53 peptide (Fig. **4**) shows that the three peptide side chains known to be essential for binding are successfully mimicked by the inhibitor. $Leu^{26}$ and $Trp^{23}$ are mimicked by the two bromophenyl groups, and $Phe^{19}$ is mimicked by the ethyl ether group. One key conclusion from this comparison is that the backbone of the natural protein ligand is simply serving as a rigid scaffold which presents key side chains in a particular spatial orientation. All the small molecule needs to do is properly mimic the key interactions made by the natural protein ligand to be an effective and potent inhibitor. The imidazoline core shows that a small molecule can, for example, economically bridge an 8-residue segment of an alpha helix.

## HCV NS3 Protease

Discovery of a macrocyclic HCV NS3 protease inhibitor, BILN 2061 (Fig. **5**), provides another illustration of the value of structural information derived from peptides [43]. Hepatitis C virus (HCV) infection is a serious cause of chronic liver disease, which can develop into life-threatening liver cirrhosis and hepatocellular carcinoma [44]. HCV is a small virus containing a single-strand RNA genome encoding a polyprotein of over 3000 amino acids [45]. The polyprotein is cleaved into four structural polypeptides by host enzymes and six nonstructural (NS) polypeptides by virally encoded enzymes, the NS2/NS3 and the NS3 proteases. Inhibition of NS3 serine protease, which is essential for viral replication [46], has proven to be a promising approach towards development of potential anti-HCV agents [47].

Lead optimization undertaken at Boehringer Ingelhein Canada was initiated from a substrate-based hexapeptide, Ac-DDIVPC-$CO_2H$, which was weakly active against the NS3 protease in presence of the synthetic NS4A peptide with an $IC_{50}$ of 39 μM. (Fig. **5**) [43]. Early SAR indicated that the introduction of a (4R)-naphthalen-1-yl-4-methoxy substituent to the P2 proline and replacing the C-terminal cysteine with the non-natural animo acid norvaline resulted in a boost

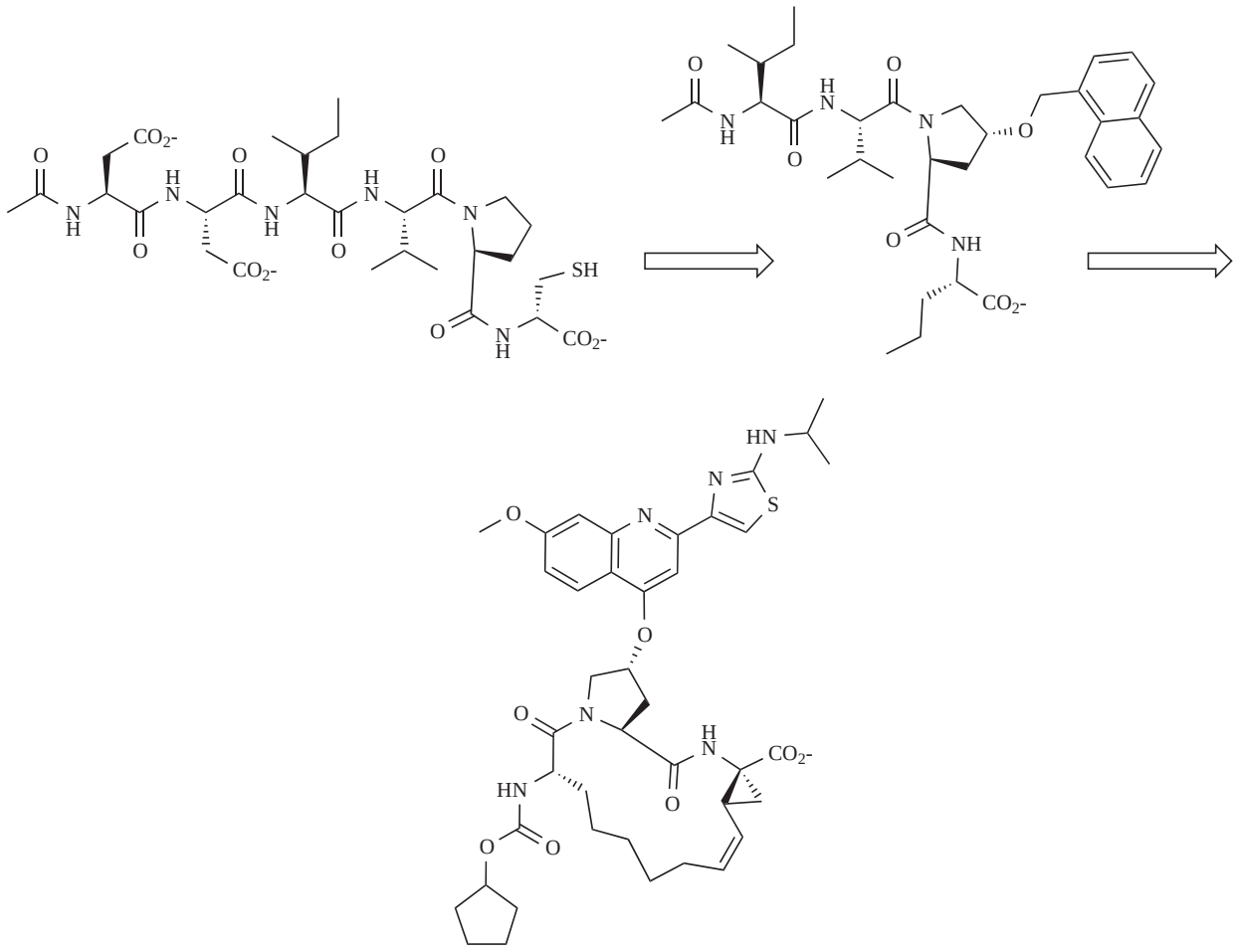


**Fig. (5).** Lead optimization from a substrate-based hexapeptide to tetrapeptide to BILN 2061.

in potency [48]. In an attempt to improve potency further and decrease the molecular size of the peptide lead, NMR line-broadening and transferred NOESY experiments were carried out [49]. The results suggested that only the four residues at the C-terminal end of the hexapeptide, designated P1 to P4, made direct interactions with the NS3 protease active site, while the two acidic residues at the N-terminus pointed toward the solvent [50]. This observation was further confirmed by transferred $^{13}C$ $T_1$ relaxation experiments on a hexapeptide [51]. However, removal of these two aspartic acids reduced the potency of the resulting tetrapeptide by 100-fold [43]. A plausible explanation to the unexpected potency loss was from the point of view of kinetics – that the electrostatic surface potential of the two acidic residues enhanced the collision rates between the peptide and protein [52]. An NMR-derived structure of the modified tetrapeptide bound to NS3 protease demonstrated that the side chain of the C-terminal norvaline curled up upon binding. This observation suggested replacing the norvaline side chain with a more rigid cyclopropyl group, and this beneficial modification was retained until the final drug candidate BILN 2061 [49]. The structure also showed that the overall binding conformation was an extended β–strand like structure. In order to reduce the inherent conformational flexibility of the linear peptide, cyclization of the tetrapeptide to mimic the β–strand was performed [53]. The NMR-derived bound conformation of the peptide, where the P3 side chain of Val was in close proximity of the P1 *n*-propyl of norvaline [50], suggested that the best strategy toward cyclization would be ring closure between these two groups. (Fig. **5**) The cyclization resulted in an inhibitor with a 15-membered macrocyclic ring, BILN 2061, which was highly potent, exhibiting an $IC_{50}$ of 28 nM. This compound has proven to be orally available [53,54].

**Integrins**

The first example of a drug-like small molecule inhibitor of a protein-protein interaction, that was designed from a peptide ligand, came from the field of integrins. Integrins are cell surface receptors that recognize a wide variety of ligands and mediate a variety of functions involving cell-cell interaction and communication. It is believed that inhibitors of integrin activity could provide benefit in diseases such as asthma, atherosclerosis, arthritis, osteoporosis, and inflammatory bowel disease, and consequently integrins have been the subject of widespread drug discovery efforts. Each integrin receptor has a heterodimeric composition, comprised of one α and one β chain, which associate non-covalently. There are at least 14 α chains and 8 β chains known, and these combine to produce over 20 different members of the integrin family.

The first family member to be targeted for drug discovery was $\alpha_{IIb}\beta_3$. This integrin receptor was known to bind ligands containing an Arg-Gly-Asp motif. The key development allowing a drug design effort was the construction of highly potent cyclic peptides containing the Arg-Gly-Asp motif. Cyclization effectively restricted the backbones so that their conformations could be accurately determined by NMR. An X-ray structure of an integrin complexed to such a ligand was not forthcoming, so the reasonable assumption was made that the constrained backbone configuration was maintained upon binding. Three groups pursued this strategy. At Genentech, the peptide used for design was (cyclo)-acetyl-(D)Tyr-Arg-Gly-Asp-Cys with the linking thioether oxidized to the sulfoxide form. Direct de novo design attempting to mimic the Arg guanidine group and the Asp carboxylate resulted in a series based on a pyrrolo-benzodiazepine-dione scaffold. These compounds attained affinities in the nanomolar range [55]. At SmithKline (now GlaxoSmithKline), design was based on the peptide: (cyclo)-2-mercaptobenzoyl-N-methyl-Arg-Gly-Asp-2-mercaptoanilide, whose structure was determined by NMR. These researchers also found that a derivatized benzodiazepine was an effective scaffold for properly positioning mimics of the Arg and Asp side chains, resulting in compounds with nM levels of activity [56]. Researchers at Lilly also started with a potent and constrained peptide - (cyclo)mercaptopropriionate-Arg-Gly-Asp-Trp-Pro-Asn. Using the NMR-derived structure as a guide, they designed inhibitors using an oxoisoquinoline scaffold. Binding affinities in the nM range were ultimately achieved [57].

The integrin designated $\alpha_v\beta_3$ also recognizes ligands posessing the Arg-Gly-Asp motif. A peptide, (cyclo)-Arg-Gly-Asp-(D)Phe-Val, had been identified that exhibited high affintiy for $\alpha_v\beta_3$, but also showed selectivty against $\alpha_{IIb}\beta_3$ [58]. A comparison of the NMR structure of this peptide was made against structures of other peptides that were potent toward both receptors. This analysis revealed that the distance between the Arg and Asp sub-pharmacophores was shorter in the $\alpha_v\beta_3$-selective peptide. Based on this information, researchers at SmithKline re-examined their benzodiazepine compounds, in particular the benzamidine substituent that was mimicking the Arg side chain. They reasoned that if the p-benzamidine had been selective for $\alpha_{IIb}\beta_3$, shortening its span by switching to the m-benzamidine might yield selectivity for $\alpha_v\beta_3$. This strategy turned out to be successful [59].

A slightly different ligand motif, namely Leu-Asp-Val, is sought by the integrin family member designated $\alpha_4\beta_1$. A cyclic peptide with potent binding activity toward this receptor was identified by a group at Tanabe Research Labs - (cyclo)-Arg-Cys-Asp-thiaPro-Cys [60]. At Roche, the structure of this peptide was determined by NMR. Remarkably, not only was the backbone rigidly constrained, but the thioproline residue was fixed completely in the cis conformation. A series of peptide analogs was designed in which the Asp-thiaPro portion was replaced with 1-(2-aminoethyl)cyclopentyl-carboxylic acid, and no loss of potency resulted [61]. By using NMR-derived structural information from the peptides, non-peptidic small molecule inhibitors were developed, based on a N-benzylpyro-glutamyl-phenylalanine core [62]. An X-ray structure of one of these small molecules suggested how it could fit to the conformation of the cyclic peptide, and what moieties were mimicking the five-membered ring system and the carboxylic acid. (Fig. **6**) This comparison allowed a strategic effort to incorporate groups to emulate the critical N-terminal Tyr residue. To provide guidance, rigid Tyr mimetics were first incorporated into versions of the cyclic peptide and structures were

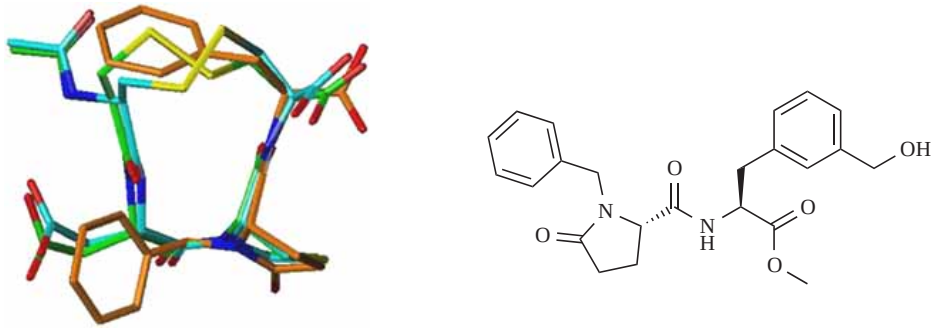



**Fig. (6).** Overlay of X-ray structure of an N-acyl-L-phenylalanine derivative (m-hydroxymethyl not shown) in gold with cyclic peptide core of Tyr-cyclo(Cys-Asp-Pro-Cys) (two conformations from the NMR-derived structural ensemble) in green and cyan.

determined by NMR. Ultimately, small molecule inhibitors of the acyl-phenylalnine class were produced that exhibited sub-nanomolar activity [63].

Recently, X-ray structures have started to become available for integrins in complex with ligands. A structure of $\alpha_v\beta_3$ has been solved with the cyclic peptide (cyclo)Arg-Gly-Asp-(D)Phe-N-methyl-Val bound [64]. It reveals that the ligand contacts both protein components of the heterodimer. The conformation of the cyclic peptide in the bound state is essentially identical to that found in the free state, as was initially hypothesized. Further, the magnesium ion which is part of a highly conserved integrin motif called the "MIDAS" domain, is coordinated to the critical Asp carboxylic acid group of the peptide. Other structures, in which $\alpha_{IIb}\beta_3$ is complexed with small molecule inhibitors, reveals a consistent binding strategy in which the basic moiety of the inhibitor is mimicking the Arg of the peptide, and the acidic moiety is mimicking the Asp [65]. This is as expected for these Arg-Gly-Asp mimetics. As for the Leu-Asp-Val mimetics from the $\alpha_4\beta_1$ system, these is no X-ray structure yet available for the complex, but it is predicted that they will similarly bridge the heterodimer, and that their carboxylic acid moieties will interact with the MIDAS motif.

## CONCLUSION

The selected cases presented herein demonstrate that peptide structure represents a highly valuable source of information for drug design. Peptides are natural substrates for many biologically important enzymes, and peptide fragments can successfully represent the binding region of a participant in a protein-protein interaction. The structures of these peptide leads can be determined in the target-bound state or, in cases where an inherent conformational propensity exists, in the free state, using the techniques of NMR and X-ray crystallography. These structures can provide guidance toward the design and optimization of compounds during a drug discovery effort. Peptides provide a unique advantage because they can simultaneously: a.) possess high potency; b.) provide a system where synthesis is relatively easy and a wide variety of building blocks is readily available; and c.) present key functional groups in a defined three-dimensional orientation that can be replicated during design of small non-peptidic mimics. This peptide-based approach offers an alternate, or adjunct, strategy to high-throughput random screening that can be highly efficient and has proven to be successful.

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