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# Comprehensive Peptidomimetic Libraries Targeting Protein–Protein Interactions

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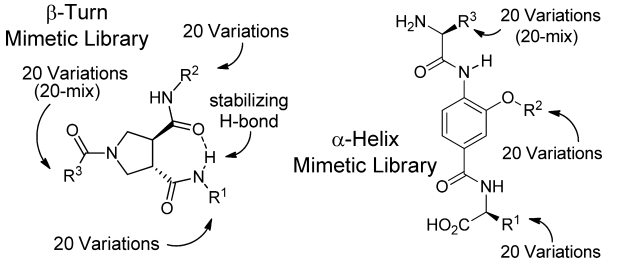
## CONSPECTUS

Transient protein–protein interactions (PPIs) are essential components in cellular signaling pathways as well as in important processes such as viral infection, replication, and immune suppression. The unknown or uncharacterized PPIs involved in such interaction networks often represent compelling therapeutic targets for drug discovery. To date, however, the main strategies for discovery of small molecule modulators of PPIs are typically limited to structurally characterized targets.




Recent developments in molecular scaffolds that mimic the side chain display of peptide secondary structures have yielded effective designs, but few screening libraries of such mimetics are available to interrogate PPI targets. We initiated a program to prepare a comprehensive small molecule library designed to mimic the three major recognition motifs that mediate PPIs (α-helix, *β*-turn, and *β*-strand). Three libraries would be built around templates designed to mimic each such secondary structure and substituted with all triplet combinations of groups representing the 20 natural amino acid side chains. When combined, the three libraries would contain a member capable of mimicking the key interaction and recognition residues of most targetable PPIs.

In this Account, we summarize the results of the design, synthesis, and validation of an 8000 member α-helix mimetic library and a 4200 member *β*-turn mimetic library. We expect that the screening of these libraries will not only provide lead structures against α-helix- or *β*-turn-mediated protein–protein or peptide–receptor interactions, even if the nature of the interaction is unknown, but also yield key insights into the recognition motif (α-helix or *β*-turn) and identify the key residues mediating the interaction. Consistent with this expectation, the screening of the libraries against p53/MDM2 and HIV-1 gp41 (α-helix mimetic library) or the opioid receptors (*β*-turn mimetic library) led to the discovery of library members expected to mimic the known endogenous ligands. These efforts led to the discovery of high-affinity α-helix mimetics ($K_i = 0.7\ \mu M$) against HIV-1 gp41 as well as high-affinity and selective $\beta$-turn mimetics ($K_i$ = 80 nM) against the $\kappa$-opioid receptor. The results suggest that the use of such comprehensive libraries of peptide secondary structure mimetics, built around effective molecular scaffolds, constitutes a powerful method of interrogating PPIs. These structures provide small molecule modulators of PPI networks for therapeutic target validation, lead compound discovery, and the identification of modulators of biological processes for further study.

## Introduction

The interaction of proteins is essential in nearly all biological pathways and processes. Transient protein–protein interactions (PPIs) form the dynamic portion of the protein interactome and are key components in signaling pathways. Aberrant signaling can be both the result and the cause of abnormal perturbations in protein interaction networks, collectively contributing to a disease phenotype.[1,2] Similarly, pathogen–host PPIs are essential to viral infection, replication, and immune suppression.[1] The PPIs involved in such interaction networks represent compelling therapeutic targets for drug discovery, but only in the past decade have they begun to gain attention as viable targets for therapeutic intervention.[3,4] Among the challenges of targeting protein interfaces are that PPIs do not bind endogenous small molecule ligands that could provide leads for discovery programs and the interfaces often present physical challenges to small molecule binding. However, the potential



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of modulating PPIs with small molecules has increased the study of how proteins interact and the interest in developing methods of targeting those interactions. A key principle to emerge is that a few key residues, referred to as "hotspot" or "anchor" residues often contribute the majority of the binding affinity to the complex.[5,6] Significantly, although endogenous small molecule ligands may not exist, the majority of PPIs are mediated by three main recognition motifs ($\alpha$-helix, $\beta$-turn, or $\beta$-strand). An attractive approach for the discovery of PPI modulators is to mimic the key interaction residues using small molecule mimetics of these recognition motifs.[7−10]

Although the field is rapidly developing, there still exists no general method capable of targeting the majority of unknown or uncharacterized PPIs. To date, five main strategies have emerged for discovery of small molecule modulators of PPIs: high-throughput screening, fragment- and peptide-based methods, computational discovery, and peptide secondary structure mimetics.[8] Fragment-based and computational methods require structurally characterized targets and mimetics of protein secondary structure and peptide-based methods are typically limited to characterized targets as well. However, recent developments in scaffolds that mimic the side chain display of peptide secondary structures have yielded effective designs that are increasingly amenable to synthetic diversification.[9,11] While there are many elegant scaffold designs,[11] few screening libraries of such mimetics currently exist that can be used to interrogate PPI targets. What is needed to tap peptide secondary structure mimetic space are effective scaffolds that are amenable to library synthesis and diversification with groups comprising the 20 natural amino acid side chains and beyond. Such a resource would constitute a powerful method for interrogating PPIs, providing small molecule modulators of PPI networks for therapeutic target validation, lead discovery, and identification of modulators of biological processes for study.

We initiated such a program to expand our library of 95 000 compounds with a comprehensive small molecule library designed to mimic the three major recognition motifs that mediate PPIs, namely, the $\alpha$-helix, $\beta$-turn, and $\beta$-strand. These three libraries built around templates designed to mimic each secondary structure and substituted with all triplet combinations of groups representing the 20 natural amino acid side chains would contain a member capable of mimicking the key interaction residues of most PPIs. The screening of this library would be expected not only to provide lead structures for most PPI targets even if the nature of the interaction is unknown, but also define the recognition motif and key residues mediating the interaction and provide a comprehensive structure−activity relationship (SAR) study for subsequent optimization. Notably, the $\beta$-turn component of this library would be particularly valuable for protein- and peptide-activated GPCRs that recognize turn structures in their endogenous ligands.[12] We present herein a summary of the design, synthesis, and validation of the first two of these libraries, an 8000-membered $\alpha$-helix mimetic library[13,14] and a 4200-member $\beta$-turn mimetic library.[15]

Enabling the production of these libraries was a solution-phase library synthesis protocol developed for generation of libraries capable of targeting protein−protein or protein−DNA interactions.[16−18] The protocol features acid/base liquid−liquid or liquid−solid extractions for the purification of products, and offers the advantages of a less limiting scale, expanded repertoire of chemical reactions, direct production of soluble intermediates and final products for assay, and lacking the linking, attachment, detachment, and capping steps required of solid phase methods. It is amenable to convergent synthetic strategies, the synthesis of mixture libraries, or use of dynamic libraries. Notably, a number of effective small molecule modulators of protein−protein[19] or protein−DNA[20,21] interactions were identified from screening these libraries.

## α-Helix Mimetic Library

**Design.** The $\alpha$-helix is the most common peptide secondary structure, constituting >40% of the polypeptide structure in proteins. An examination of the multiprotein complexes in the Protein Data Bank concluded that 62% of these complexes feature a helix at the interaction interface.[22] Our design for the $\alpha$-helix mimetic library template was based on the terphenyl scaffold **1** and subsequent oligoamide scaffold **2** introduced by Hamilton and co-workers (Figure 1).[23] These designs constitute a rigid framework from which *o*-substituents are projected to mimic the side chains at the *i*, *i*+4, and *i*+7 positions of an $\alpha$-helix. We envisioned an extension of the Hamilton design to triaryl amide scaffold **3**,[13,24,25] where the subunits are joined by an amide coupling reaction subject to purification by acid/base extraction, and the *o*-alkoxy substituent side chain diversification could be achieved by a well established aromatic substitution reaction of 3-fluoro-4-nitrobenzoate in which the activating nitro substituent additionally serves as a "protected" nitrogen for eventual coupling.

**Testing and Refinement of the α-Helix Mimetic Design against MDM2/p53.** The well-characterized intracellular

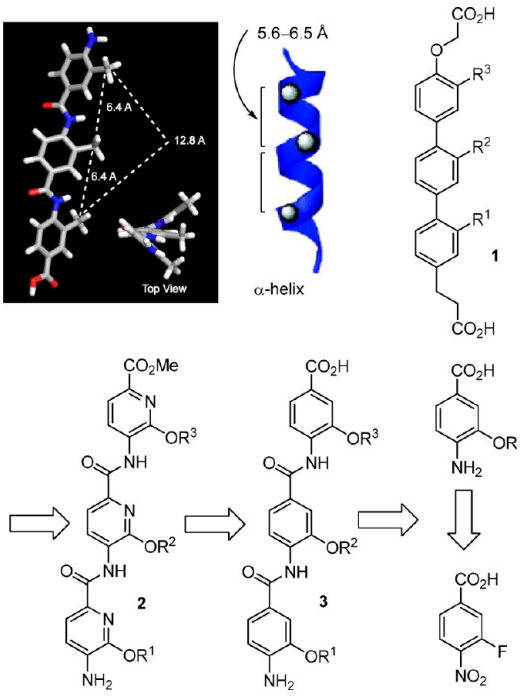



**FIGURE 1.** Initial design of the α-helix mimetic library template based on extension of the terphenyl scaffold introduced by Hamilton.

MDM2/p53 PPI was chosen as the target against which the α-helix design would be refined. The MDM2/p53 interaction has attracted considerable attention because of its therapeutic potential for the treatment of tumors with disregulated p53 resulting from overexpression of MDM2.[26] The X-ray structure of a bound p53 peptide revealed a well-defined MDM2 hydrophobic binding pocket that is occupied by three key amino acid side chains (Phe[19], Trp[23], Leu[26]) on one face of a p53 α-helix.[27]

Ten iterative variants on the template **3** were prepared (80 compounds) and examined for inhibition of MDM2/p53 binding (Figure 2), in which the substituents were chosen to mimic the p53 α-helix Phe[19], Trp[23], and Leu[26] side chains. The number of unnatural aryl subunits (1−3), the position of the aryl alkoxy substituent (3-alkoxy vs 2-alkoxy), the order of the side chain presentation (e.g., [Phe]-[Trp]-[Leu] vs [Leu]-[Trp]-[Phe]), and the incorporation of a Nap versus Trp central side chain were examined (Figure 2). From this assessment, the modified template **4** emerged as the most effective where the sequential incorporation of the natural amino acids at the termini improved activity, suggesting that the spatially more rigid side chain presentation embodied in the triaryl template is improved with the more flexible natural amino acids that may adjust the projected side chain distances. Additionally, the physical properties of the compounds, especially their water solubility, improved as the

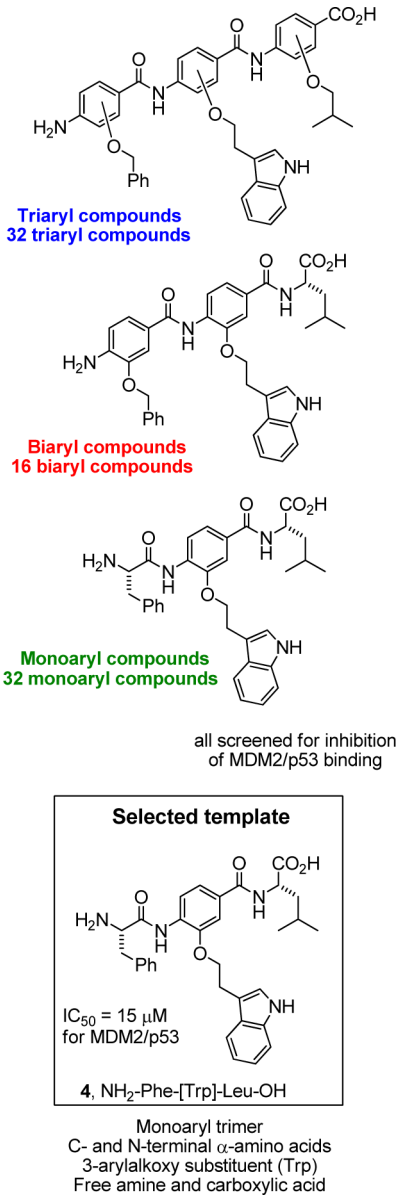
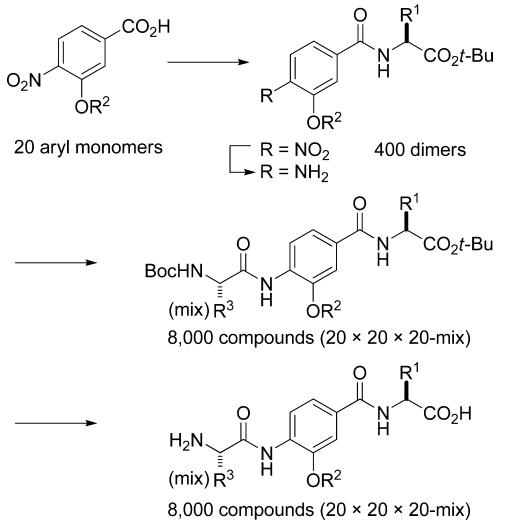



**FIGURE 2.** Candidate p53/MDM2 inhibitors prepared and screened in the α-helix mimetic library template selection process.

number of aryl subunits was reduced as did their synthetic complexity. These features coupled with the activity of the modified design led to its selection for library synthesis. Finally, the concern that the terminal carboxylic acid and free amine might preclude cell permeability in cellular assays can be addressed by utilizing the final, still protected library (Scheme 1).

**Synthesis of the α-Helix Mimetic Library.** The approach used for the construction of the library is outlined in Scheme 1. By introducing 20 amino acid side chain variants at each position of the trimer scaffold and with the provision that reactive (Cys), structural (Pro), and redundant (Gln, Glu) amino acid side chains were replaced by those known to

**SCHEME 1.** Format and Synthesis for the Library




dominate interactions with proteins (Nap, Phe(4Cl), HoPhe, TyrMe), all possible combinations produce 8000 compounds representing all permutations on a naturally occurring α-helix. Consistent with the screening capabilities of academic collaborators, the library was assembled as 400 mixtures of 20 compounds (20 × 20 × 20-mix), conducting the final coupling with a full mixture of the 20 amino acids. The identification of the compounds responsible for any mixture activity is conducted by resynthesis of the individual 20 compounds in the mixture from archived samples of the precursor dimers (one step) and their individual rescreening. Facilitating the systematically optimized library synthesis, the isolation and purification of each intermediate and final product were conducted by acid/base liquid–liquid extractions and both the identity of the products and their purity were established.

**Screening the Library Against MDM2/p53.** The library (400 wells) was screened for inhibition of MDM2/p53 binding. Consistent with expectations and representative of the immediate informative results available through examination of the library, the data from the 400-well screening consolidated in Figure 3A revealed that the most effective central subunit was Trp. The screening also revealed that it was the central subunit versus the C-terminal residue that dominated the MDM2/p53 inhibitory activity, which is consistent with reports that identified Trp[23] as the primary anchor residue in the interaction. Further examination of the 20 C-terminal residues for the central subunit Trp mixtures revealed that Leu is preferred with significant activity observed for the closely related aliphatic residues Abu and Val, as well as the bulky aromatic residues Nap and Phe(4Cl)

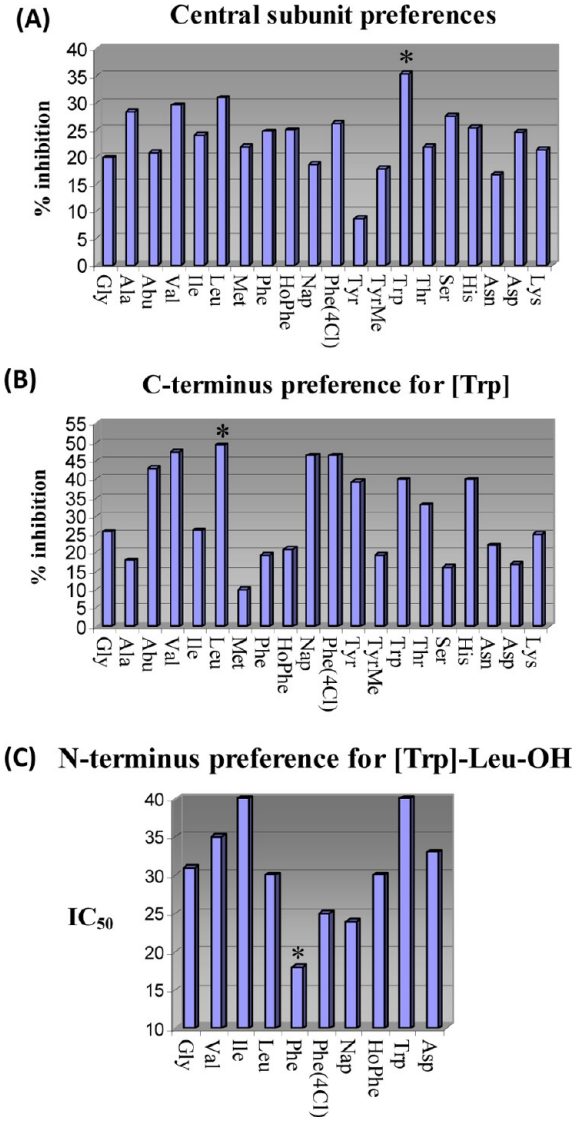



**FIGURE 3.** (A) Central subunit preferences, average % inhibition of MDM2/p53 binding for the 20 mixtures containing the defined central subunit. (B) C-terminus preferences for [Trp], % inhibition of MDM2/p53 binding for the 20 compound mixtures $H_2N$-mix-[Trp]-XXX-OH. (C) $IC_{50}$ values for the inhibition of MDM2/p53 binding by individual compounds $H_2N$-XXX-[Trp]-Leu-OH with $IC_{50} < 40$ $\mu$M.

(Figure 3B). Deconvolution by resynthesis of the individual members of the $H_2N$-XXX-[Trp]-Leu-OH mixture and their assessment revealed that Phe is preferred at the N-terminus over the other 19 residues, followed by the closely related aromatic residues Phe(4Cl) and Nap (Figure 3C). Thus, the library screening and subsequent deconvolution led to the discovery of the lead inhibitor $H_2N$-Phe-[Trp]-Leu-OH used to define the α-helix mimetic screening library template. The screening therefore identified the known primary anchor residue and produced the expected lead structure, establishing the effective α-helix mimicry of the template and

suggesting that information on a PPI target may be confidently extrapolated from library screening results.

**Validation of the α-Helix Mimetic Library against HIV-1 gp41.** The HIV-1 envelope glycoprotein gp41 has emerged as a promising viral cell entry inhibitor target because it contains a deep hydrophobic pocket in the N-heptad repeat (NHR) region that is critical to formation of the fusogenic state and amenable to small molecule binding.[28−30] The interaction of the C-heptad repeat (CHR) with the NHR hydrophobic pocket is a challenging intramolecular PPI mediated principally by Trp$^{628}$, Trp$^{631}$, Asp$^{632}$, and Ile$^{635}$ on the CHR α-helix with the Trp residues constituting the anchor residues (Figure 4). The hydrophobic residues at the *i*, *i*+3, and *i*+7 positions of the α-helix project downward into the NHR pocket, while Asp$^{632}$ makes an electrostatic interaction with Lys$^{574}$ at the pocket periphery. Because of the therapeutic potential and α-helical nature of the interaction, the gp41 hydrophobic pocket is an attractive target for small molecule α-helix mimetics.[31−33]

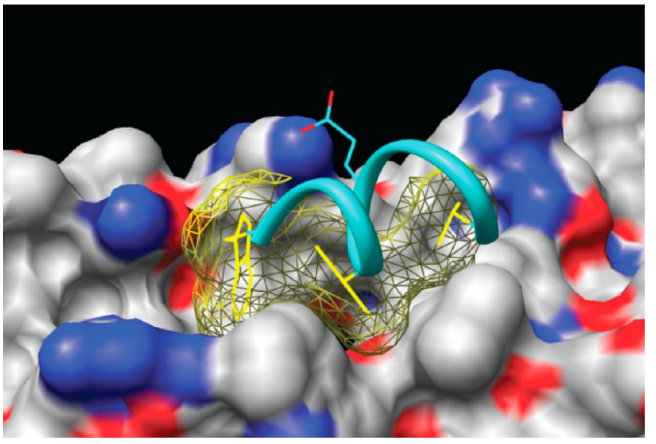


**FIGURE 4.** The hydrophobic pocket of gp41 (modeled structure, R. Rizzo). The CHR hydrophobic (Trp628, Trp631, and Ile635, in yellow) and charged (Asp632, in cyan) residues are shown in the pocket.

With use of a metallopeptide-based assay designed to directly detect inhibitors that bind the NHR pocket,[34] we screened the α-helix mimetic library.[35] The fractional fluorescence values from the library screening were used to compose a primary screen SAR (Figure 5A). The most active mixtures contained the [Tyr], [Phe(4Cl)], [Nap], and [Trp] central subunits and the strongest activity was observed when these dominant central aromatic side chains were combined with aromatic or bulky aliphatic residues at the C-terminus. The Ile side chain ranked highest among the C-terminal residues, in line with expectations for mimicry of the CHR Trp-Trp-Ile helical face (Figure 5C). Good activity was also observed with other bulky aliphatic (Leu, Met) or a variety of aromatic (HoPhe, Nap, Phe(4Cl), or TyrMe) residues in the C-terminal position, although their impact is more muted than the dominant central subunit.

Consistent with the expected mimicry of the CHR α-helix, the individual compounds in the XXX-[Nap]-HoPhe, XXX-[Phe(4Cl)]-HoPhe, and XXX-[Trp]-Leu series displayed a

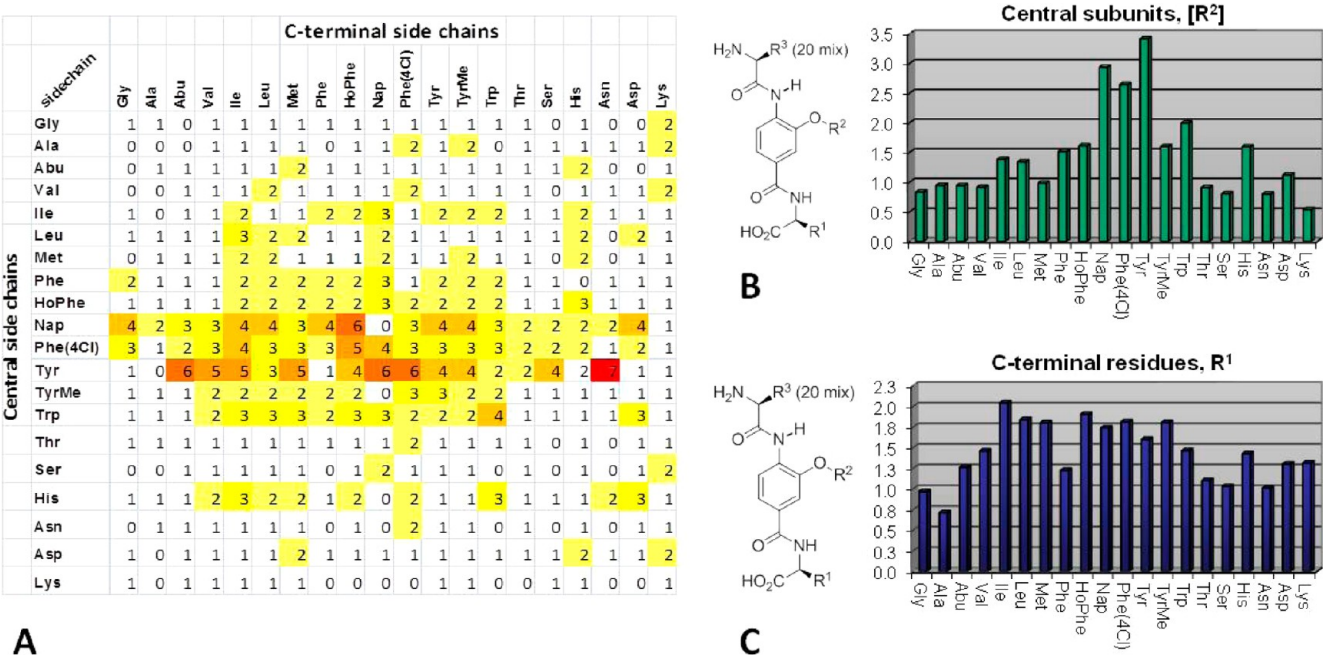



**FIGURE 5.** (A) 20 × 20 matrix of fractional fluorescence values (×10) from the binding assay on the 400 α-helix mimetic library mixtures. (B) The sum average scores of individual central subunits. (C) The sum average scores of C-terminal residues.

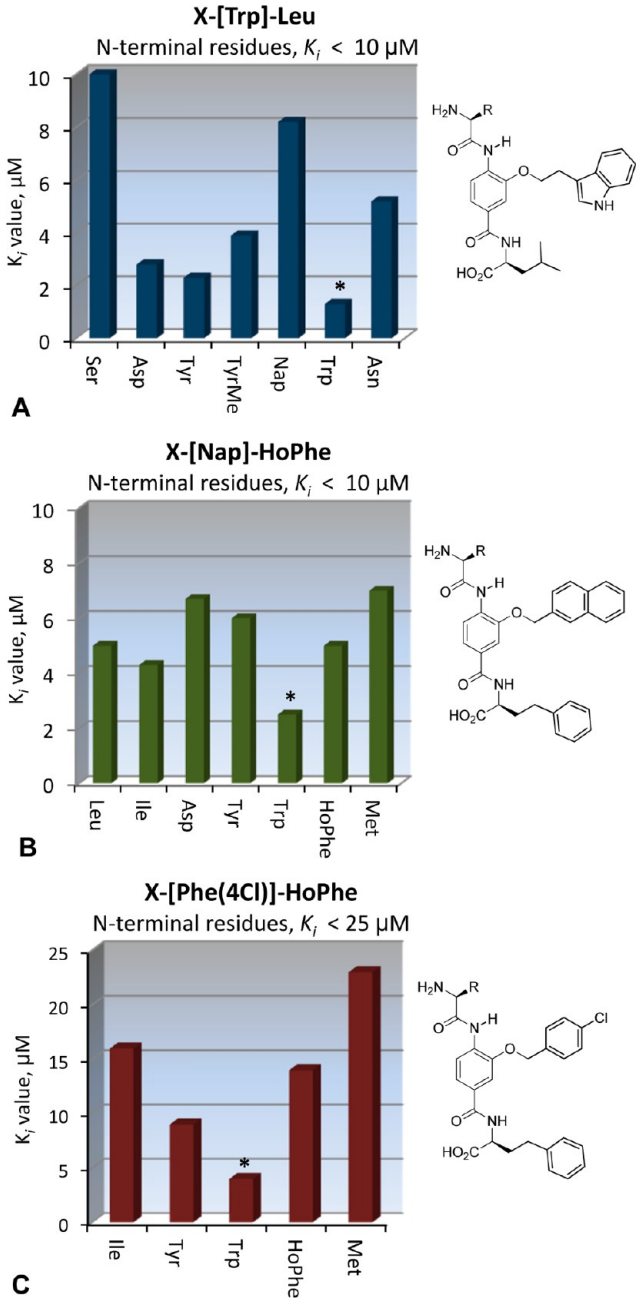



**FIGURE 6.** $K_i$ values from the binding assay conducted on 20 individual compounds in each series. Those with $K_i > 10$ or 25 $\mu$M not shown.

strong N-terminal preference for Trp, mimicking the Trp[628] interaction, Figures 6 and 7. The most active compounds in these series presumably mimic the Trp-Trp-Ile helical face with the central [Trp], [Nap], or [Phe(4Cl)] subunits and the C-terminal Leu or HoPhe residues mimicking Trp[631] and Ile[635], respectively, to yield compounds with $K_i$'s of 1−4 $\mu$M. The most potent compound in the three series was $H_2N$-Trp-[Trp]-Leu-OH ($K_i = 1.3$ $\mu$M), representing the identification of the expected binding partner from the library (Figure 6A). Presumably, an N- to C-terminal orientation is facilitated by an

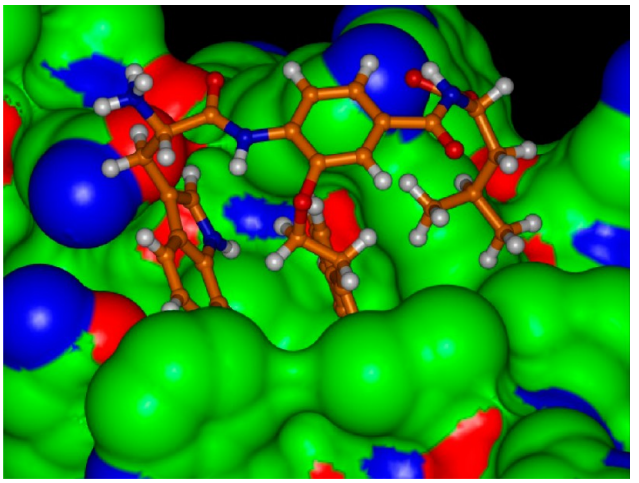


**FIGURE 7.** Proposed binding mode for $H_2N$-Trp-[Trp]-Leu-OH.

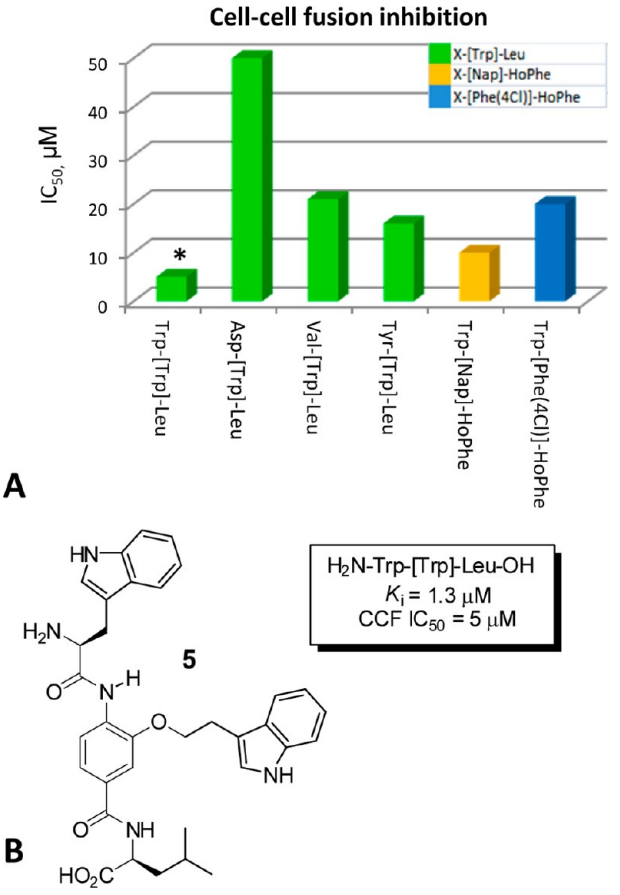



**FIGURE 8.** (A) $IC_{50}$'s of most active individual compounds in CCF assay. (B) New lead compound discovered.

electrostatic interaction between their terminal carboxylic acid and Lys[574] on the NHR pocket periphery, mimicking the endogenous interaction of Asp[632] (Figure 7).

Analysis of single compounds in a functional cell−cell fusion (CCF) assay (Figure 8A) demonstrated that the most

potent fusion inhibitor was $H_2N$-Trp-[Trp]-Leu-OH ($IC_{50}$ 5 $\mu$M), whose sequence corresponds to the key CHR α-helix residues. The library evaluation therefore identified the aromatic [Trp], [Phe(4Cl)], [Nap], and [Tyr] central subunits as the dominant feature in active mixtures, and Ile emerged as the best C-terminal residue. The individual compounds in the XXX-[Nap]-HoPhe, XXX-[Phe(4Cl)]-HoPhe, and XXX-[Trp]-Leu series displayed an N-terminal preference for Trp, indicating that they mimic the Trp-Trp-Ile helical face bound in an N- to C-terminal orientation. The most potent compound in the series examined was $H_2N$-Trp-[Trp]-Leu-OH (**5**, $K_i$ = 1.3 $\mu$M, CCF $IC_{50}$ = 5 $\mu$M), and it corresponds to the key CHR α-helix interaction residues. Thus, the α-helix mimetic library directly provided a new lead structure (Figure 8B) that matches or exceeds the potency of small molecules discovered over the past decade, validating its α-helix mimicry and the power it possesses for interrogating PPIs.

## β-Turn Mimetic Library

**Design.** The β-turn is one of the three main secondary structural motifs found in proteins and peptides and occurs where the polypeptide strand reverses direction. Because β-turns are often defined structures found on the surface of proteins, they are frequently key interaction motifs in PPIs. A closely related class of interactions in which turn structures are important is the peptide−receptor interactions, the best characterized example being the interaction of the peptide-activated G protein-coupled receptors (GPCRs) with their endogenous peptide ligands. Although the peptide ligands of this subset of GPCRs are flexible and adopt variable secondary structures, the active conformation recognized by the receptor typically involves a turn structure (β- or γ-turn).[12]

An ideal β-turn mimetic library scaffold would be constrained to approximate the geometric display of the amino acid side chains found within a β-turn, be sufficiently flexible to allow the side chains to approximate the side chain vectors of the many turn types, and be amenable to library synthesis with groups representing the 20 natural amino acids. To facilitate template discovery, we employed a geometric analysis of the mean distances found between α-carbon centers in a set of 10 245 β-turns in the protein data bank (Figure 9).[36]

Recognizing that one of the turn amino acids ($i$+1 or $i$+2) often serves a structural rather than a recognition role (e.g., Pro or Gly),[36] we elected to mimic Cα triplets in which either $C\alpha_{i+1}$ or $C\alpha_{i+2}$ is omitted. From this analysis, we found that *trans*-pyrrolidine-3,4-dicarboxamide could serve as a synthetically accessible template on which to display the side chain groups (Figure 10A). Rigidified by an intramolecular

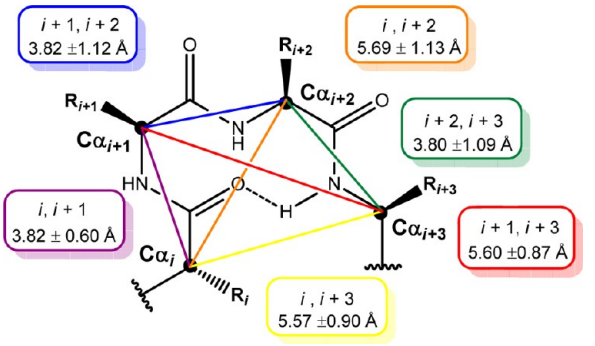



**FIGURE 9.** Mean and standard deviations of the Cα distances taken from a set of 10 245 β-turns in the PDB.

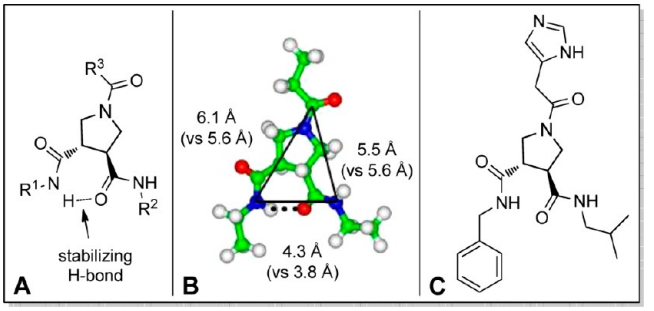



**FIGURE 10.** (A) The *trans*-pyrrolidine-3,4-dicarboxamide template. (B) Low-energy conformation and measured distances between substituted centers of a *trans*-pyrrolidine-3,4-dicarboxamide with ethyl substituents. (C) A *trans*-pyrrolidine-3,4-dicarboxamide substituted with groups representing the side chains of Leu, Phe, and His.

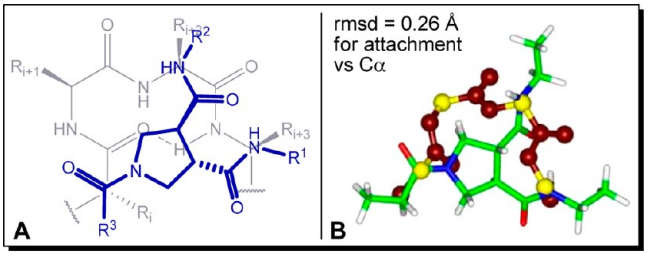



**FIGURE 11.** (A) β-turn mimicry by a trisubstituted *trans*-pyrrolidine-3,4-dicarboxamide. (B) Overlay and calculated rmsd value for attachment of the three substituted centers of the template with three α-carbons in a type I β-turn.

H-bond, the lowest energy conformation of the *trans*-pyrrolidine-3,4-dicarboxamide has substituted centers that conform closely to the triangle geometries of the Cα triplets in which either $C\alpha_{i+1}$ or $C\alpha_{i+2}$ is omitted (Figure 10B). An overlay of the low-energy conformation with the peptide backbone of a type I β-turn (Figure 11) demonstrated a potential mode of mimicry and yielded an rmsd value of 0.26 Å for ethyl-substituted centers versus the Cα triplet $C\alpha_i$, $C\alpha_{i+2}$, $C\alpha_{i+3}$. In line with the design, the flexible noncovalent constraints that stabilize the

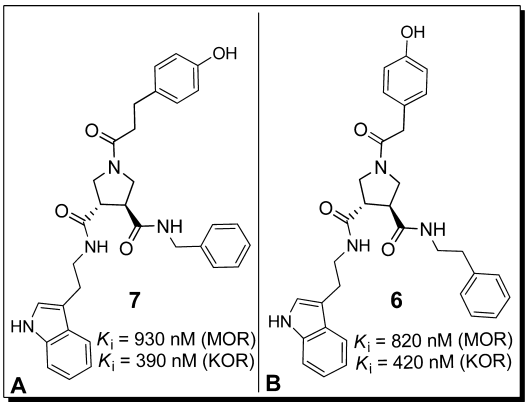


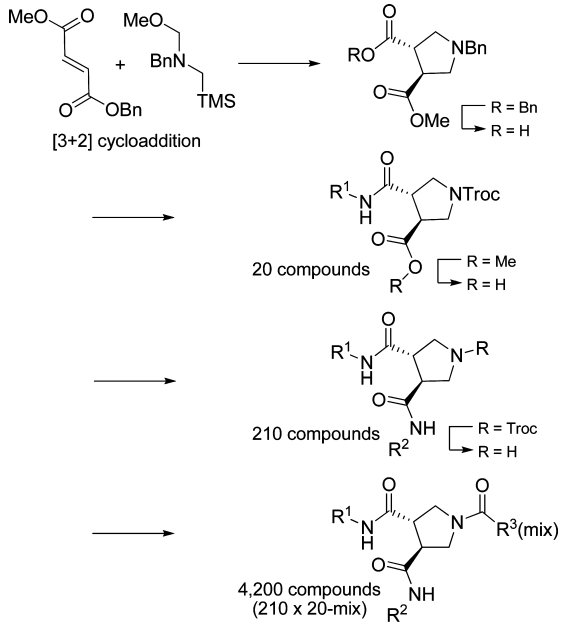

**FIGURE 12.** Structures of KOR-selective compounds **6** and **7**.

lowest energy conformer also permit the compound to adopt variable H-bond donor/acceptor patterns and permit the attached side chains to approximate the vector display in a range of $\beta$-turn structures. Significantly, the template can be substituted using amide coupling reactions and possesses a simplifying $C_2$ symmetry axis that allows all triplet combinations of 20 side chains to be accomplished with only 4200 compounds (vs 8000).

**Validation of the Design against Peptide-Activated GPCRs: The Opioid Receptors.** In order to establish the ability of substituted *trans*-pyrrolidine-3,4-dicarboxamides to modulate $\beta$-turn mediated recognition events, a series of compounds was evaluated against a representative set of peptide-activated GPCRs that recognize $\beta$-turns in their endogenous ligands. The $\mu$-opioid receptor (MOR) is a well-known clinical target for the treatment of pain. While MOR selective opiate analgesics such as morphine remain the drugs of choice for the treatment of severe pain, their use is limited by well-characterized side effects. The additional two members of the opioid receptor family, the $\kappa$-opioid and $\delta$-opioid receptors (KOR and DOR), have also been investigated as analgesic targets. In particular, targeting of peripheral KOR has emerged as a promising treatment for inflammatory and visceral pain as well as arthritis.[37]

Two selective endogenous peptide ligands of the opioid receptors are endomorphin-1 (H-Tyr-Pro-Trp-Phe-$NH_2$) and endomorphin-2 (H-Tyr-Pro-Phe-Phe-$NH_2$).[38] There is considerable evidence that the active conformation of the endomorphins for MOR is a $\beta$-turn, including activities observed with analogues that incorporate turn constraints and appropriately substituted $\beta$-turn mimetics.[9,39,40] We prepared a series of pyrrolidine-3,4-dicarboxamides designed to mimic the three side chain residues in the pharmacophore of the endomorphins along with several Ala negative controls and measured their binding to MOR, KOR, and DOR (Figure 12).

**SCHEME 2**




The highest affinity for the MOR was exhibited by compound **6** with the $R^1/R^2$ combination of Trp/HoPhe and $R^3$ of Tyr ($K_i = 820$ nM) followed by compound **7** with $R^1/R^2$ of Trp/Phe and $R^3$ of HoTyr ($K_i = 930$ nM). In addition to exhibiting submicromolar activity using a simple template, the activity reflected a combination of side chains found in the endogenous ligands. Moreover, the compounds demonstrated the best affinity to MOR when single carbon extensions of key side chains (HoPhe or HoTyr) were incorporated, and this influenced the selection of side chains incorporated into the library. Interestingly, the compounds showed even higher affinities for the KOR than for the MOR, highlighted by **7** and **6** with $K_i$ values measured at 390 and 420 nM, respectively (Figure 12).

**Synthesis of the $\beta$-Turn Mimetic Library.** Convinced that the pyrrolidine-3,4-dicarboxamide template would be a valuable $\beta$-turn mimetic, the synthesis of the comprehensive $\beta$-turn mimetic library was conducted, Scheme 2. The order of the side chain introductions (amide couplings; $R^1$, $R^2$, and $R^3$) and the location of the 20-mix functionalization ($R^3$) were dictated by the $C_2$ symmetry of the template and the simplifying opportunity it presented for the number of compounds required to represent all 20 $\times$ 20 $\times$ 20-mix combinations (210 individual $R^1/R^2$ combinations $\times$ 20-mix for $R^3$). Facilitating the systematically optimized library synthesis, the isolation and purification of each intermediate and the racemic final products were conducted by acid/base liquid–liquid extractions, and both the identity of the products and their purity were established.

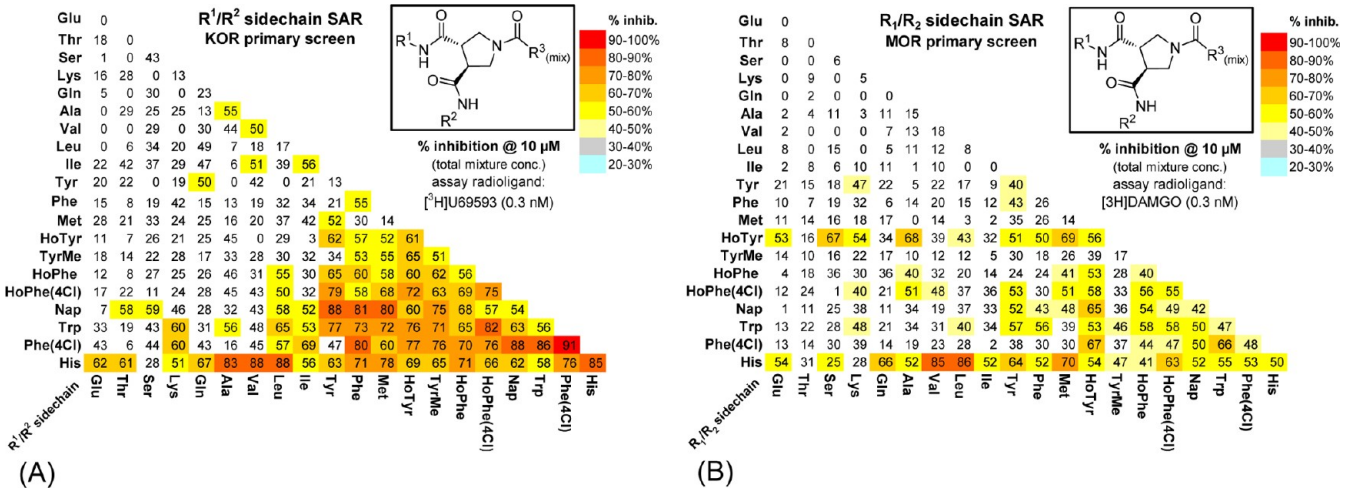



**FIGURE 13.** (A) Percent inhibition of [$^3$H]U69593 binding for the indicated $R^1/R^2$ side chain combinations against the KOR. (B) Percent inhibition of [$^3$H]DAMGO binding for the indicated $R^1/R^2$ side chain combinations against the MOR.

**Screening the Library Against the Opioid Receptors.** The complete library was screened at 10 $\mu$M (0.5 $\mu$M/compound) for activity at human cloned opioid receptors (KOR, MOR, and DOR), and the screening results for the KOR and MOR are shown in Figure 13. The overall trends against the three receptors proved consistent with the initial single compound results in exhibiting an intrinsic binding selectivity for the KOR, followed by the MOR and then DOR. A clear SAR trend emerged where hydrophobic aromatic side chains dominate the interaction with the opioid receptors, with Phe(4Cl), Trp, and Nap showing strong inhibition against the KOR and the HoTyr, Trp, and HoPhe(4Cl) side chains ranking among the best against the MOR.

**Deconvolution and Analysis of Individual Compounds.** Based on the screening data and the $\beta$-turn sequence of the endomorphins (H-Tyr-Pro-Trp/Phe-Phe-$NH_2$), three mixtures were selected for deconvolution (Figure 14). The Trp/Phe series is the closest representative within the library of the endomorphin-1 sequence and contains compound **7**, which exhibited $K_i$ values of 390 and 930 nM for KOR and MOR, respectively. The Trp/Phe(4Cl) series is closely related, but the addition of the 4-Cl substituent enhanced binding of the mixture to both the KOR and the MOR (Figure 14). The Phe(4Cl)/Phe(4Cl) series chosen for deconvolution was the mixture that displayed the highest affinity against the KOR (91% inhibition) and the greatest difference between KOR and MOR affinity (91% vs 48%). These mixtures were deconvoluted by resynthesis of the individual compounds from the archived penultimate intermediates.

The 60 compounds were screened at 10 $\mu$M for activity at the KOR and MOR via radioligand binding assays. Consistent

| $R^1/R^2$ sidechains | % inhibition KOR | MOR | DOR |
|---|---|---|---|
| Trp/Phe | 73 | 56 | 25 |
| Trp/Phe(4Cl) | 86 | 66 | 26 |
| Phe(4Cl)/Phe(4Cl) | 91 | 48 | 18 |

**FIGURE 14.** Mixtures selected for deconvolution.

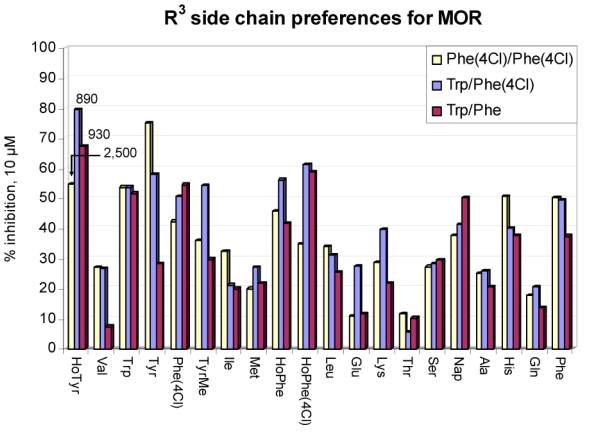



**FIGURE 15.** Screening results (% inhibition at 10 $\mu$M) for the three series of compounds against MOR along with measured $K_i$ values (nM) (vs [$^3$H]DAMGO).

with expectations, the screening against the MOR demonstrated that either HoTyr or Tyr was the favored $R^3$ substituent in all three series, with HoTyr performing best in the Trp/Phe and Trp/Phe(4Cl) series and Tyr yielding the most potent compound in the Phe(4Cl)/Phe(4Cl) series (Figure 15). Beautifully, and if the turn recognition motif were unknown, these results would represent the identification of the Tyr-XXX-Trp-Phe and Tyr-XXX-Phe-Phe $\beta$-turn motifs.

The data from the three deconvolution series is shown in Figure 16. Clear from these comparisons is the greatest

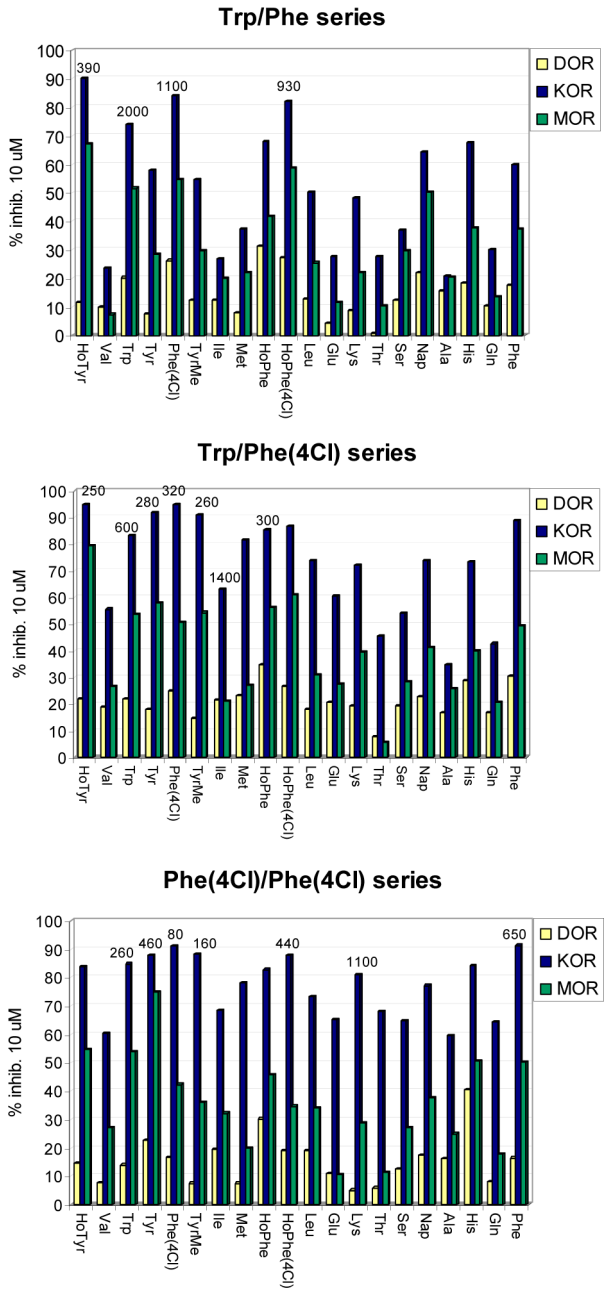



**FIGURE 16.** Screening results (% inhibition at 10 $\mu$M) for the three series of compounds against KOR (vs [$^3$H]U69593), MOR (vs [$^3$H]DAMGO), and DOR (vs [$^3$H]DADLE) along with measured $K_i$ values (nM) against KOR.

activity against the KOR (KOR > MOR > DOR) for all three series and that the activity of the three series generally follows the order of Phe(4Cl)/Phe(4Cl) > Trp/Phe(4Cl) > Trp/Phe as observed in the mixture screening results (Figure 13). The activity of **7** ($K_i$ = 390 nM) used originally to test the design was improved with the replacement of Phe with Phe(4Cl) in the Trp/Phe(4Cl) series providing **8** ($K_i$ = 250 nM, Figure 17A), and this series provided several related compounds with $K_i$'s of <300 nM. The most active

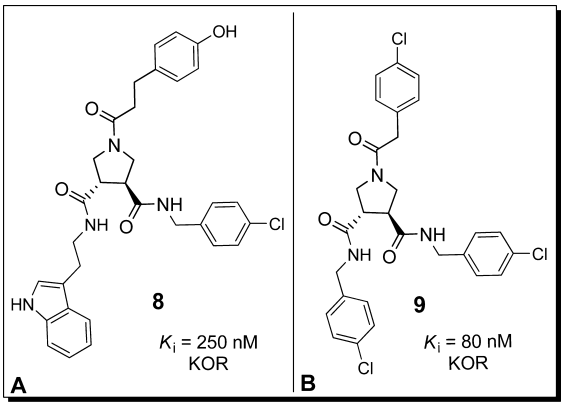



**FIGURE 17.** Structures of **8** and **9**.

Phe(4Cl)/Phe(4Cl) series not only showed a greater activity for the KOR, but it also exhibited a greater selectivity for the KOR versus the MOR or DOR as inferred from the original screening results (Figure 13). The most potent compound in this series for the KOR was **9** ($K_i$ = 80 nM, Figure 17B), which was found to be >100-fold selective versus the MOR or DOR ($K_i$ >10 000 nM).

## Conclusion

As key components of a comprehensive small molecule library targeting the major recognition motifs of PPIs ($\alpha$-helix, $\beta$-turn, $\beta$-strand), we summarized the results of the design, synthesis, and validation of an 8000 member $\alpha$-helix mimetic library and a 4200 member $\beta$-turn mimetic library. The library templates were designed to mimic the respective peptide secondary structure and to support synthetic diversification with triplet combinations of groups representing the 20 natural amino acid side chains. The templates were refined and validated by the screening of individual compounds against characterized $\alpha$-helix- or $\beta$-turn-mediated interactions. Upon identification of satisfactory templates, the libraries were assembled using a solution phase protocol with liquid−liquid extractions for purification and conducted on a scale that insures their long-term availability for screening campaigns. The screening of these libraries is expected not only to provide lead structures against $\alpha$-helix- or $\beta$-turn-mediated protein−protein or peptide−receptor interactions, even if the nature of the interaction is unknown, but also to yield insights into the recognition motif ($\alpha$-helix or $\beta$-turn), and identify the key residues mediating the interaction. Consistent with this expectation, their screening against p53/MDM2 and HIV-1 gp41 ($\alpha$-helix mimetic library) or the opioid receptors ($\beta$-turn mimetic library) led to the discovery of library members expected to mimic the endogenous ligands. These efforts also led to the discovery of high-affinity $\alpha$-helix

mimetics ($K_i$ = 0.7 $\mu$M) against HIV-1 gp41 as well as high-affinity and selective $\beta$-turn mimetics ($K_i$ = 80 nM) against the $\kappa$-opioid receptor. It was demonstrated that the SAR generated by the screening of the libraries not only provides insights into the identity of the key interaction residues but also provides valuable information on binding preferences and modes of interaction that can facilitate subsequent lead optimization. The studies, therefore, suggest that the use of such comprehensive libraries of peptide secondary structure mimetics, built around effective molecular scaffolds, constitutes a powerful method of interrogating PPIs, providing small molecule modulators of PPI networks for therapeutic target validation, and lead discovery. When combined with libraries designed to target protein−DNA interactions[20,41] and the major untapped enzyme classes,[42,43] they provide a powerful paradigm for discovery or validation of new therapeutic targets using small molecules.

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## FOOTNOTES

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The authors declare no competing financial interest.

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