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Molecular design of small organic molecules based on structural information for a conformationally constrained peptide that binds to G-CSF receptor

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ABSTRACT

Based on structural information for a peptide (P8-2KAQ) that binds to granulocyte-colony stimulating factor receptor (G-CSFR), small ligands with a biaryl scaffold were designed and their binding affinities were evaluated.

Despite intense research into the design of small ligands that target protein–protein interaction interfaces, a novel methodology for the rational design of such ligands remains an elusive goal. To design such ligands, our group has examined the directed evolution of peptides in a phage-displayed library of conformationally constrained peptides. Screening of the library against targeted proteins should provide bioactive peptides whose rigid structures indicate the required spatial orientation of the pharmacophores, thus facilitating structure-based design of peptidomimetics. In a previous study, we constructed a library of de novo designed helix-loop-helix peptides (35 amino acids).[1] This structural motif allows polypeptides to form stable α-helices[2] that often present recognition sequences in biological processes.[3]

We have applied our method to ligand design of granulocyte-colony stimulating factor (G-CSF) receptor that an important cytokine receptor stimulating bone marrow to release granulocytes and stem cells into the blood.[4] The phage-displayed library was screened to give a binding peptide, **P8-2KAQ** (**1**). Here, we report the design and synthesis of small molecule ligands for the receptor, based on structural information of the helix-loop-helix peptide.

The binding affinity of **P8-2KAQ** (**1**) was found to be dependent on the amino acids leucine, lysine, glutamic acid and glutamine on the α-helix (*i*, *i*+2, *i*+4 and *i*+5). Virtual screening which took into account the spatial orientations of these amino acid residues suggested that *N*-benzyl aniline and biphenyl frameworks would be suitable scaffolds for peptidomimetics of this helix-loop-helix peptide. Thus, we designed and synthesized the four ligands listed in Figure 1. The design of these ligands took into account simulations of all the amino acid side chains involved in the binding of the active peptide with G-CSF receptor. *N*-Benzyl aniline derivative ligand **2** was synthesized first (Scheme 1). Commercially available 3-nitrocinnamic acid **6** was treated with water soluble carbodiimide (WSCI), 1-hydroxybenzotriazole (HOBT) and aqueous ammonia to give amide **7** in 55% yield. Both the double bond and the nitro group of **7** were reduced by hydrogenation to give arylamine **8** in 91% yield. Reductive amination of **8** with aldehyde **9** using $NaBH_3CN$ gave *N*-benzyl aniline **10**. *N*-Benzyl aniline **10** was subjected to a second reductive amination and subsequent hydrolysis to afford acid **11**. Finally, **11** was hydrolyzed using TFA to give *N*-benzyl aniline ligand **2** in 88% yield.

Synthesis of another *N*-benzyl aniline derivative ligand **3** is depicted in Scheme 2. Reductive amination of 3-bromoaniline with aldehyde **9** afforded the secondary amine **13** in 91% yield. Next, a second reductive amination of **13** with methyl 3-oxopropanoate and subsequent hydrolysis by NaOH furnished acid **14** in 85% yield. *N*-Benzyl aniline **14** was treated with WSCI, HOBT and aqueous ammonia to give amide **15** in 87% yield. Amide **15** was subjected to the Mizoroki–Heck reaction[5] with methyl acrylate, affording **16** in 55% yield. Next, the reduction of the double bond in **16** gave

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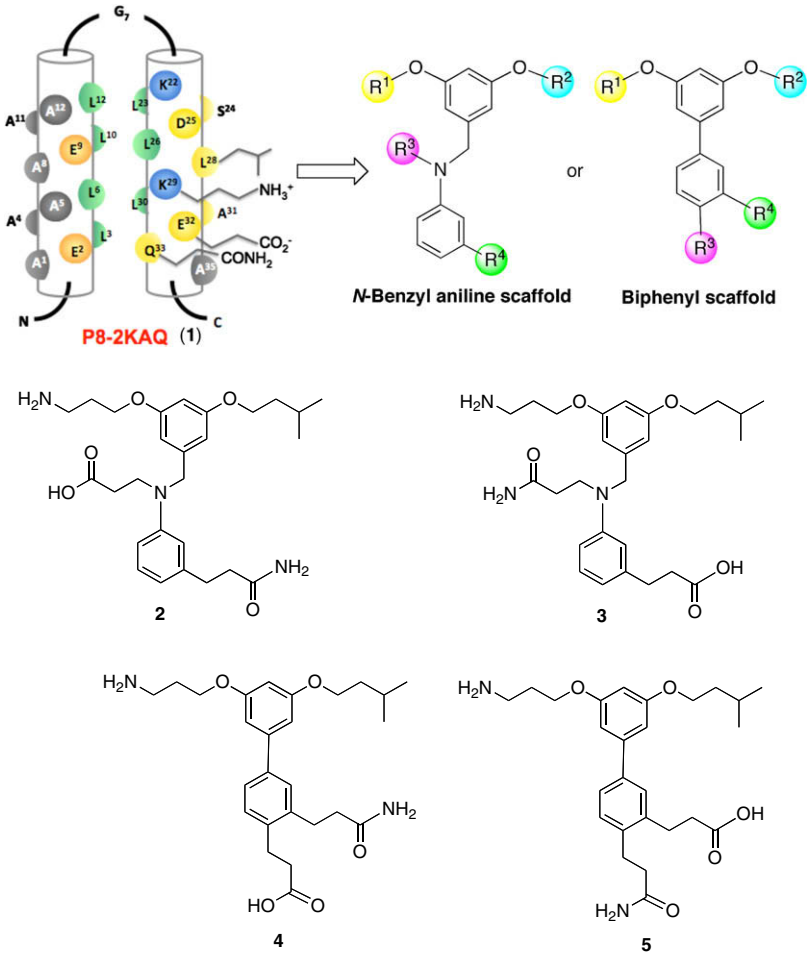
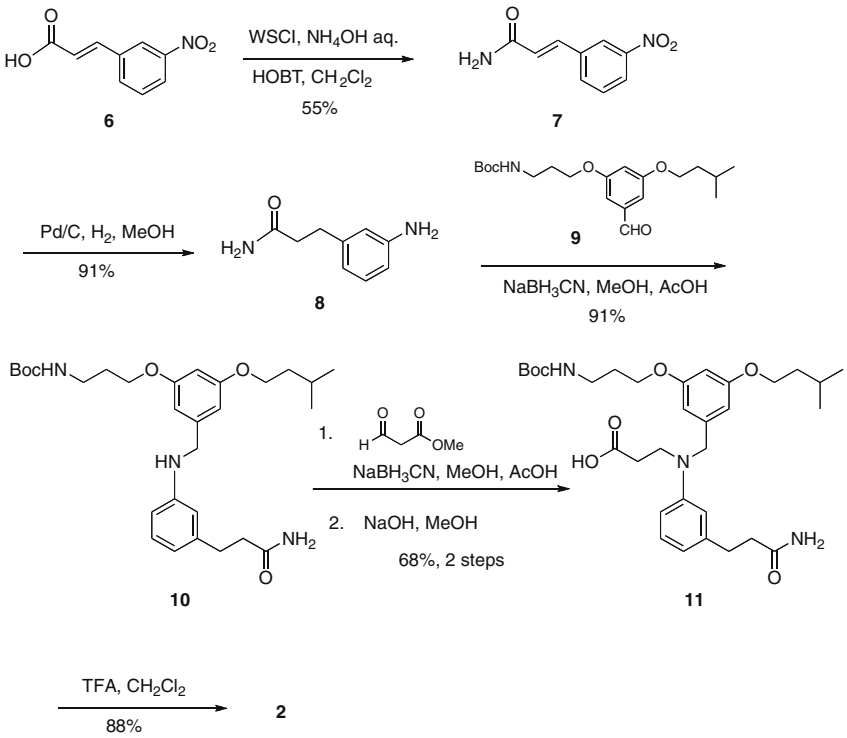
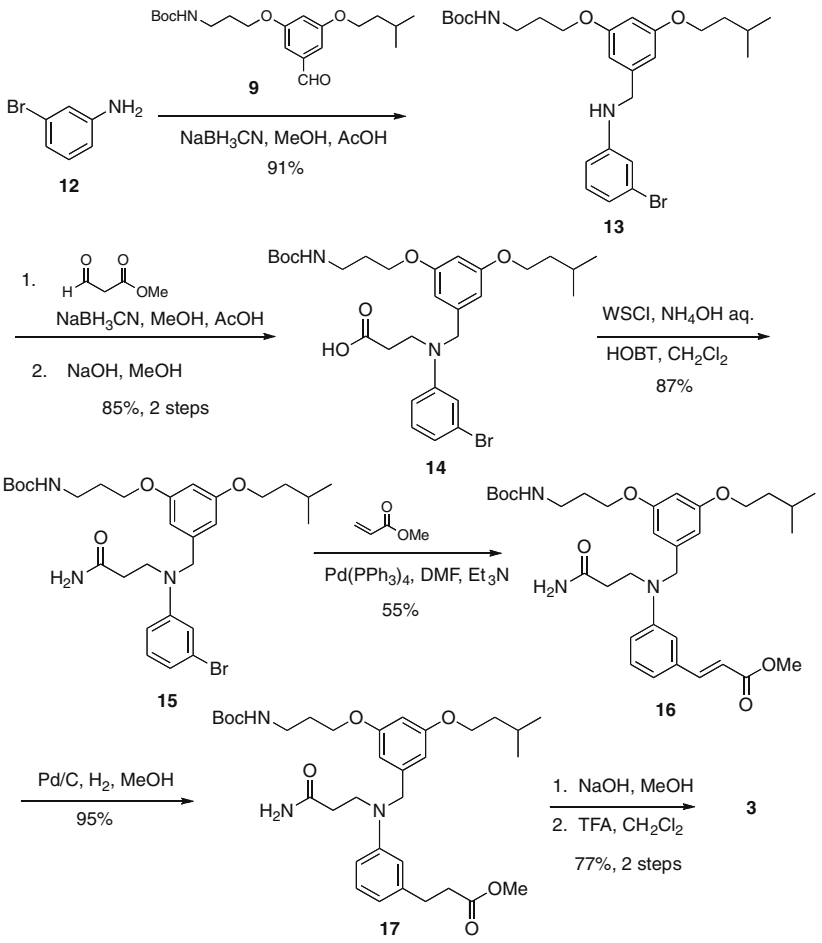


Figure 1. Design of tetrasubstituted ligands.



Scheme 1. Synthesis of *N*-benzyl aniline ligand **2**.



Scheme 2. Synthesis of *N*-benzyl aniline ligand **3**.

17. Completion of the requisite *N*-benzyl aniline ligand **3** was achieved by ester hydrolysis and then deprotection.

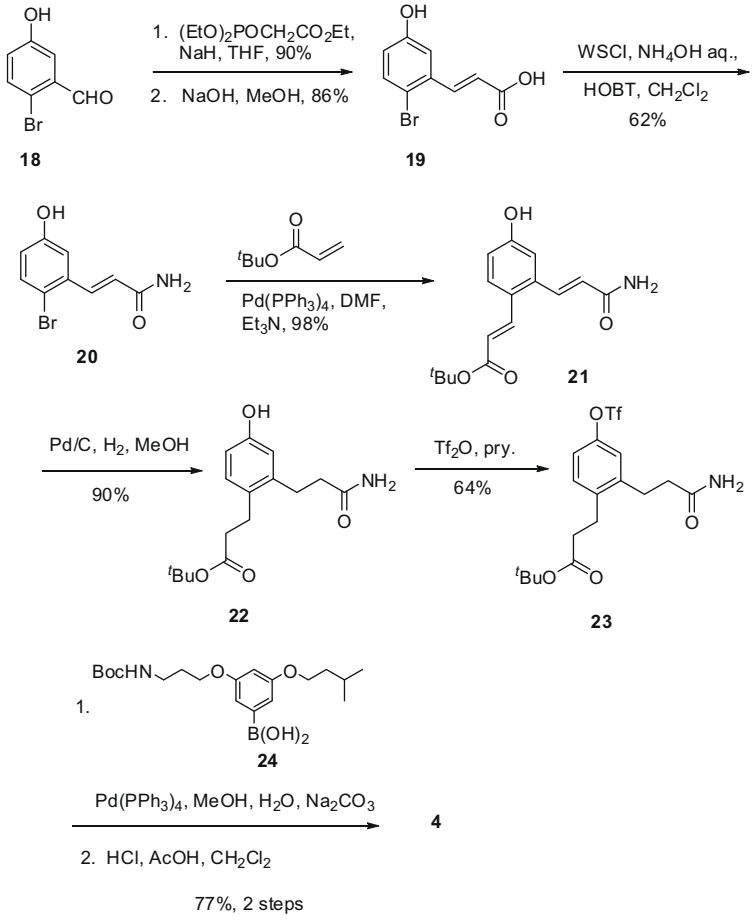
We next examined the synthesis of biphenyl ligands. Hamilton and co-workers reported that a terphenyl scaffold could reasonably mimic functionality in a spatial orientation, involving side chains of the *i*, *i*+3 and *i*+7 residues on an α-helix.[6] In our case, the important pharmacophores of the G-CSFR binding peptide are enshrined on the amino acid residues of *i*, *i*+2, *i*+4 and *i*+5 on the α-helix. Although the positions of the targeted residues are different, it would be probable that the biphenyl framework can also act as an effective scaffold for the design of small ligands. Therefore, we designed and synthesized tetrasubstituted biaryl ligands **4** and **5** with four residues attached at the 3-, 3′-, 4′- and 5-positions.

The synthesis of biphenyl ligand **4** is shown in Scheme 3. 2-Bromo-5-hydroxybenzaldehyde **18**[7] was first converted to an α,β-unsaturated ester by Horner–Wadsworth–Emmons olefination, followed by treatment with NaOH/MeOH to afford acid **19**. Treatment of **19** with WSCI, HOBT, and aqueous ammonia provided the corresponding amide **20** in 62% yield. Amide **20** was reacted with *t*-butyl acrylate using the Mizoroki–Heck protocol to give α,β-unsaturated ester **21**. Next, two double bonds in **21** were reduced by hydrogenation to give phenol **22**. Phenol **22** was transformed into trifilate **23** in moderate yield (68%). Trifilate **23** was coupled with boronic acid **24** under Suzuki–Miyaura cross-coupling conditions[8] to give the biphenyl coupling product. Subsequently, deprotection of the biphenyl compound using acetic acid pre-saturated with hydrochloric acid afforded the target ligand **4** in 77% yield over two steps. A second biphenyl ligand **5** was also synthesized using a similar procedure.[9]

The binding affinities of ligands **2**–**5** for G-CSF receptor were evaluated using surface plasmon resonance (SPR) biosensor techniques, which directly provide information regarding the characteristics of small molecule–protein interactions, and allow real-time monitoring of the interactions (Table 1).[10] Initially, *N*-benzyl aniline ligand **2** showed promising binding affinity for G-CSF receptor, with a dissociation constant (K_d) of 47 μM (entry 1). As expected from the design, replacement of the functional groups at positions R^3 and R^4 resulted in total loss of binding affinity (entry 2, ligand **3**). Biphenyl ligand **4** bound to G-CSF receptor with a K_d of 20 μM (entry 3). On the other hand, the replacement of functional groups in the biphenyl scaffold resulted in loss of binding affinity (entry 4, ligand **5**), similar to that with the *N*-benzyl aniline scaffold.

Thus, the position of the functional groups strictly affects affinity in both *N*-benzyl aniline and biphenyl ligands. These observations suggest that the observed binding affinity is due to specific interactions between the ligands and the receptor, and are not due to nonspecific hydrophobic interactions.

In conclusion, we designed and synthesized small molecules binding to G-CSF receptor based on the structure of a conformationally constrained helix-loop-helix peptide. Both *N*-benzyl aniline and biphenyl scaffolds were found to be candidates for small compounds binding to G-CSF receptor. The interaction of G-CSF with its receptor stimulates survival, proliferation, differentiation and function of neutrophil precursors and mature neutrophils.[11]



Scheme 3. Synthesis of biphenyl ligand **4**.

Table 1
Dissociation constants of ligands **2**–**5** determined by SPR

Entry	Ligand	K_d (μM)
1	**2**	47
2	**3**	–[a]
3	**4**	20
4	**5**	–[a]

[a] No binding affinity.

Therefore, ligands inhibiting the interaction would be of interest as cytotoxic agents for the treatment of cancer.[12] Further design of new ligands are ongoing in an effort to delineate the activity of this class of small compound.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.010.

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