Development of a Pharmacophore Model for a Novel Hematoregulatory Peptide

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Hematopoietic growth factors play a pivotal role in orchestrating cellular host defense mechanisms against bacterial, fungal, and viral infections. The utility of these agents lies in their ability to stimulate nonspecific host defense by increasing immune cell numbers and enhancing the host effector cell function.[1] Although protein therapeutics have proven beneficial, their chronic administration still poses challenges such as immunogenicity and oral delivery. Therefore, structurally simple, low molecular weight compounds that either mimic or induce endogenous growth factors are attractive targets for drug design. We have reported on the peptide **1** (SK&F 107647, Figure 1) that stimulates proliferation of murine and human granulocyte−macrophage colony-forming units (CFU-GM) in vivo, enhances bone marrow stem cell engraftment during bone marrow transplant, and increases antimicrobial activity of macrophages and polymorphonuclear (PMN) cells.[2] In infection models, SK&F 107647 (**1**) enhances the survival of mice infected with either *Candida albicans* or Herpes simplex type 2 virus.[3,4] Prophylactic administration of **1** (both alone and in combination with antibiotics) to rats infected with a fibrin−thrombin clot containing either Gram-negative (*Escherichia coli*) or Gram-positive (*Streptomyces aureus*) bacteria enhances survival.[5] SK&F 107647 (**1**) manifests its biological response by inducing hematoregulatory growth factors from bone marrow stromal cells.[6]

The paucity of information about the putative receptor for **1** has limited our ability to rationally design non-peptidic or peptidomimetic analogs for this compound. A detailed structure−activity relationship (SAR) study of **1** and its analogs has shown quite specific requirements for biological activity in the colony-stimulating activity (CSA) assay.[2] The C-terminal carboxylates of **1** can be replaced with carboxamides without loss of activity, and Glu3 can be replaced with Asp or Ser suggesting that a charged side chain is not required at this position. There is a stringent requirement for an acidic residue at position 3 where Asp was replaceable only with Glu. This suggests that this residue probably forms a critical ionic interaction with a complementry basic residue. The amino group of lysine at position 5 as well as its spatial location is critical for the biological activity, since analogs containing lower homologs of lysine, such as ornithine and 2,4-diaminobutyric acid, or any other amino acid were virtually inactive at the highest dose tested (1 mg/mL). This again suggests that the Lys5 residue is involved in a critical ionic interaction.

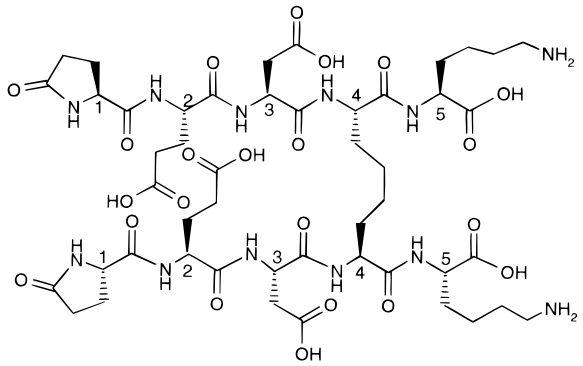

Figure 1. Structure and numbering system for SK&F 107647 (**1**).

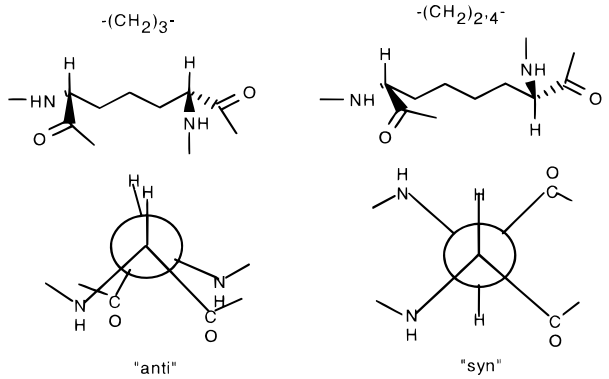

Figure 2. Schematic of a,a′ substituents on the odd- and even-membered "methylene bridge" containing residue at position 4.

The SAR for the spacer (position 4), connecting the two halves of the molecule, is even more remarkable. The number of methylene units spanning the diaminodicarboxylic acids at position 4 is critical. The di- and tetramethylene spacers (diaminosuberic acid and diaminoadipic acid) are well tolerated; whereas, the mono-, tri-, penta-, and hexamethylene spacers (diaminoglutaric acid, diaminopimelic acid, diaminoazelaic acid, and diaminosebacic acid) are not. If one assumes that the bridge adopts an extended conformation, an even-membered alkylene spacer could place the two peptide chains in a "syn" relationship, while an odd-membered spacer would orient them in an "anti" relationship (Figure 2). It is possible that this type of conformational bias plays a role in determining the biological activity of a given analog. The vast difference in the EC_{50} of analogs containing even- and odd-membered spacers suggests that the distance and the relative orientation the two peptide chains are crucial for biological activity.

Considering this data, we propose a model where the side chains of the Asp3 and Lys5 residues are involved in intramolecular ionic interactions creating salt bridges and, along with the methylene spacer of residue 4, present residues 1 and 2 to the putative receptor. Two possible permutation of the model which include intramolecular salt bridges are shown in Figure 3. To test this model, the peptide analog **2** was synthesized where both the Asp3and Lys5 were replaced with Glu3and Orn5. These modifications change the length of the side chains of both residues 3 and 5 without affecting the overall size of the pseudoring formed by the intramolecular salt bridges. Analog

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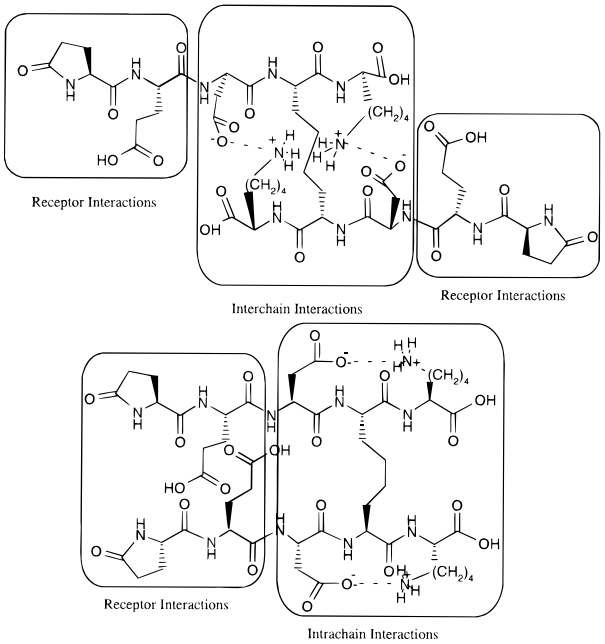
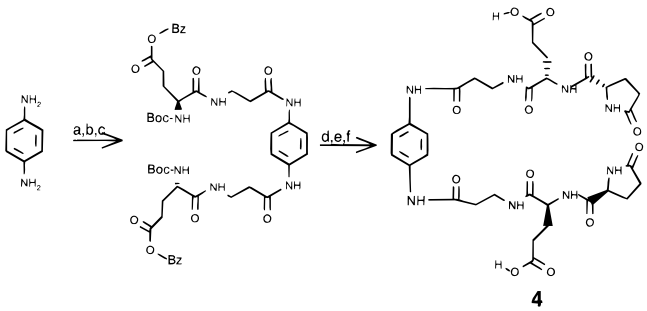


Figure 3. Hypothetical models for the side chain interactions of **1**.

Scheme 1. Scheme for the Synthesis of Compound **2**[a]



[a] (a) Boc-β-Ala, EDC·HCl, HOBt, DIEA, DMF. (b) TFA, CH_2Cl_2. (c) Boc-Glu(OBz), BOP, HOBt, DIEA, DMF. (d) TFA, CH_2Cl_2. (e) pGlu, BOP, HOBt, DIEA, DMF. (f) HF, 0 °C.

2 was shown to be equipotent to **1**, while analog **3**, which contains a smaller pseudoring structure, was inactive. These results are consistent with the proposed model where the side chains of residues 3 and 5 interact ionically to form salt bridges (either intrapeptide chain or interpeptide chain) with each other, locking a biologically active conformation (Figure 3).

We reasoned that if residues 3−5 serve to lock a specific biologically active conformation and present residues 1 and 2 in the correct orientation, then it should be possible to replace this entire scaffold with a simpler structure. To test this hypothesis a series of compounds was synthesized in which residues 3−5 were excised and replaced with various spacers. While most of these changes resulted in inactive molecules, incorporation of *N*,*N*′-bis(β-alanyl)-1,4-diaminobenzene as a spacer yielded a biologically active peptidomimetic analog **4** (Scheme 1). Molecular modeling experiments (data not shown) showed that low energy conformations of this spacer would be able to accommodate either the "parallel" or "antiparallel" pharmacophore model (Figure 3).

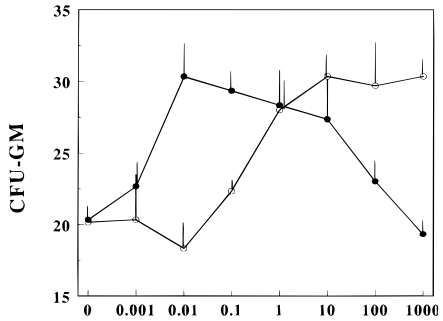


Figure 4. Colony-stimulating activity of compound **1** [●] and compound 4 [○] on C6.4 cells.

Table 1. EC_{50} of **1** and Its Analogs in the CSA Assay

compound	structure[a]	EC_{50} (pM)
1	(pGlu-Glu-Asp)$_2$-Sub-(Lys-OH)$_2$	4
2	(pGlu-Glu-Glu)$_2$-Sub-(Orn-OH)$_2$	4
3	(pGlu-Glu-Asp)$_2$-Sub-(Orn-OH)$_2$	na[b]
4	(pGlu-Glu-β-Ala)$_2$-Dab	684

[a] Sub = (2-*S*,7-*S*)-2,7-diaminosuberic acid; Dab** = 1,4-diaminobenzene. [b] na not active at 1 mg/mL.

The novel peptidomimetic **4**, designed on the basis of this hypothesis, represents a major improvement over the structure of **1**. Evaluation of **4** in the CSA assay indicated that this compound retains 100% of activity of **1** (Figure 4). Although the potency of **4** does not match the potency of **1**, it is still a very potent hematoregulatory molecule with an EC_{50} in subnanomolar range (Table 1). This data is especially impressive since over half of the molecular mass and six asymmetric centers have been eliminated. These results further validate the pharmacophore model and allow for the refinement of our knowledge of the recognition event. Specifically, the results support that residues 1 and 2 are important for the recognition event and that residues 3−5 serve to orient residues 1 and 2 into their biologically active conformation. These data also imply that the achiral spacer of **4** (a) may not be the optimal replacement for residues 3−5 and (b) is conformationally too flexible to allow differentiation of the "antiparallel" or "parallel" pharmacophore models.

In conclusion, we have proposed a pharmacophore model for the interaction of SK&F 107647 (**1**) and its analogs with their putative "receptor". This model is supported by the data on several key peptide analogs. In addition, we have designed and synthesized a biologically active peptidomimetic analog validating many aspects of the proposed model. These results show that it is possible to use this model to rationally design structurally simple, low molecular weight molecules that manifest many of the biological effects seen by hematopoietic growth factors.

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Supporting Information Available: Experimental details and characterization data (9 pages). See any current masthead page for ordering and Internet access instructions.

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