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Novel Peptidomimetic Hematoregulatory Compounds

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Abstract—The activity of a novel series of peptidomimetic hematoregulatory compounds, designed based on a pharmacophore model inferred from the structure–activity relationships of a peptide SK&F 107647 (**1**), is reported. These compounds induce a hematopoietic synergistic factor (HSF) which in turn modulates host defense. The compounds may represent novel therapeutic agents in the area of hematoregulation.

Introduction

Hematopoietic growth factors such as G-CSF, M-CSF, GM-CSF and EPO have received considerable attention in recent years.[1–3] These factors play key roles in regulating cellular host defense mechanisms involved in combating bacterial, fungal and viral infections.[4] They stimulate host defense mechanisms by both increasing immunocompetent cell numbers and enhancing effector cell functions. Although these proteinaceous factors are clinically effective,[5–8] a small molecule capable of mimicking the effects of these factors may complement existing immunomodulatory therapy.

We have recently reported the activity of a low molecular weight peptide, SK&F 107647 (**1**) which stimulates hematopoiesis (hematopoiesis is the process whereby stem cells, proliferate and differentiate into mature blood cells, for reviews, see refs 9 and 10) in vitro and in vivo (Fig. 1).[11,12] We have also formulated a pharmacophore model associated with **1** which was used to design and synthesize a structurally simpler, biologically active, peptidomimetic analogue, **7**.[13] The model states that the pGlu-Glu subunits of **1** represent the pharmacophore of this compound and that the remaining structural elements of **1** function as a scaffold to correctly present the pharmacophore to its putative receptor. Implicit in this model is the fact that the scaffold in **1** should not be unique. This model has also been used by Cuthbertson et al. to design an alternative peptidomimetic analogue.[14] In this paper we describe the in vitro activity of a series of analogues based on **7**, as well as the in vivo activity of a selected analogue **5a**.

Chemistry

Compounds **5a–e**, **7** and **9** (Table 1) were synthesized as outlined in Scheme 1. 1,4-Phenylenediamine dihydrochloride was di-acylated with Boc-β-Ala using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and hydroxybenzotriazole hydrate (HOBt) in the presence of *i*-Pr_2NEt. The Boc-protecting groups were removed by exposure to trifluoroacetic acid (TFA) and the resulting diamine was acylated with either Boc-Ser(Bn) or Boc-Glu(OBn) using benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent) and HOBt in the presence of *i*-Pr_2NEt to give **3** and **6**, respectively. Compound **3** was treated with TFA and the resulting diamine was acylated with the acids shown to give **4a–e**. Racemic azetidine-2-carboxylic acid was used to synthesize **4d** giving diastereomeric products which were easily separated by flash chromatography. No attempt was made to assign the absolute stereochemistry of the azetidine ring of these products. Exposure of compounds **4a–e** to anhydrous

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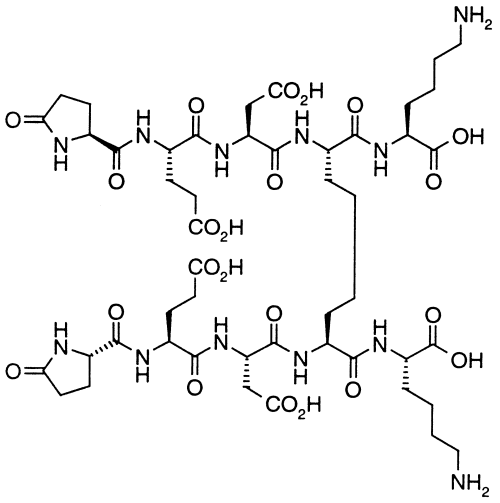

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Figure 1. Structure of SK&F 107647 (**1**).

Table 1. In vitro hematopoietic synergistic factor (HSF) released by 0.1, 1.0 and 10.0 μg/mL of **1**, **5a**–**e**, **7**, and **9**

No.	R	0.10 μg/mL	1.0 μg/mL	10.0 μg/mL
1		2.5×10e2 ± 4.4e1	2.1×10e4 ± 1.1e4	1.4×10e6 ± 8.7e5
5a		1.5×10e2 ± 1e3	2.1×10e3 ± 4.5e2	1.0×10e4 ± 8e2
5b		2.3×10E2 ± 8.1e1	1.5×10E3 ± 9e2	1.4×10E4 ± 1.1e4
5c		1.4×10E2 ± 8.6e1	1.1×10E3 ± 9e2	1.5×10E4 ± 8.4e3
5d		2.7×10E2 ± 1.2e2	4.4×10E3 ± 9e2	1.1×10E4 ± 9.3e3
5e		ND[a]	2.2×10E2 ± 8.3e1	2.0×10E3 ± 8.2e2
7		1.6×10e2 ± 8.3e1	1.8×10e3 ± 8e2	1.5×10E4 ± 1.2e4
9		1.4×10E2 ± 1.2e2	1.5×10E3 ± 1e3	1.7×10E4 ± 1.1e4

[a]ND, not detected.

HF gave, after purification, **5a**–**e**, which were used for biological evaluation. Compound **6** was treated with TFA and then acylated with pGlu. Exposure to anhydrous HF gave **7**.

Compound **9** was prepared in a similar fashion to the analogues described above except that Fmoc-*N*-Me-Ser(Bn) was used instead of Boc-Ser(Bn) or Boc-Glu(OBn) to give **8**. The Fmoc-protecting group of **8** was removed with piperidine and the resulting diamine was acylated with picolinic acid using BOP reagent and HOBt in the presence of *i*-Pr_2NEt. Exposure of this compound to anhydrous HF gave **9**.

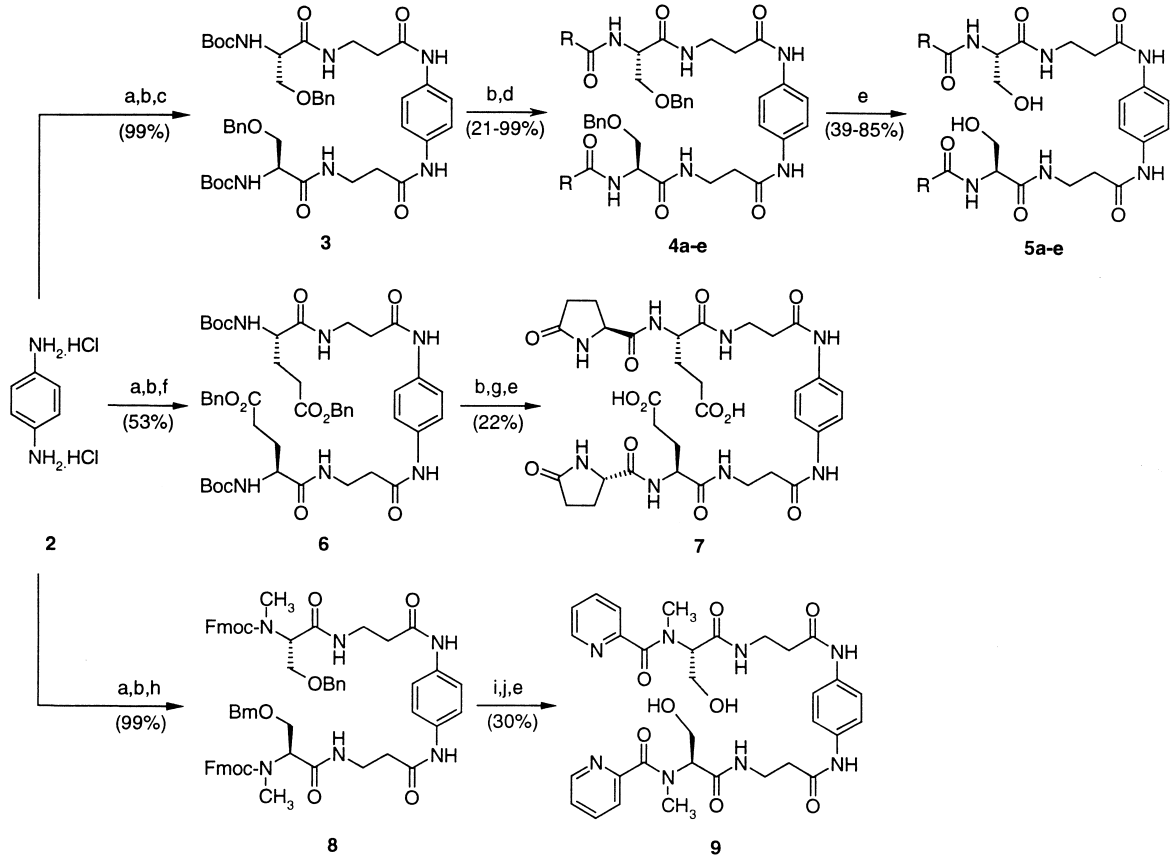
Biology

The test compounds were evaluated in vitro for their ability to induce a hematopoietic synergistic factor (HSF), from a murine stromal cell line (C6.4).[15,16] The induced HSF synergizes with endogenous myelopoietic growth factors (G-, M-, GM-CSF) and augments the differentiation and maturation of stem cells from bone marrow, resulting in an increase in progenitor cells (CFU-GM) in vitro. The amount of HSF activity induced was determined using a murine granulocyte-macrophage colony-forming unit (CFU-GM) assay.[17] The HSF activity of the stromal cell supernatants exhibited a distinct 'bell-shaped' dose response curve.[12,13] Inactive compounds were defined by the lack of induced HSF activity at the doses tested (0.10, 1.0 and 10.0 μg/mL of test compounds). Using this assay, we determined which structural changes were tolerated in this series of compounds. However, due to the lack of a receptor binding assay, it is difficult to quantitatively assess the effects of relatively modest structural changes on the interaction of a given analogue with its putative receptor. The previously described analogue **1**, was included in all the assays at a single dose (1.0 μg/mL) as a positive control.

Compound **5a** was tested in vivo in a murine model of infection.[18] Mice were treated ip with either compound **5a** or dilution buffer control. Two hours after treatment, the mice were challenged iv with a lethal dose of *C. albicans* and followed for survival.

Results and Discussion

The pyroglutamic acid and glutamic acid moieties of **7** could be replaced by picolinic acid and serine to give **5a**. This matches the selectivity seen for the peptide SAR around **1**. The picolinoyl groups of **5a** could be replaced by small nitrogen-containing heterocyclic carboxylic acids (**5b**–**e**). Substituting dehydroproline (**5b**), proline (**5c**) or azetidine carbonyl (**5d**) for the picolinoyl group in **5a** give compounds that are equipotent in the HSF assay, whereas, substitution with the pyrrolyl group results in a 10-fold loss in potency. Furyl and thiophenyl groups were not acceptable substitutions for the picolinoyl groups of **5a**. The location of the nitrogen in the heterocyclic ring with respect to the amide was also found to be important. Only rings containing nitrogen alpha to the carbonyl group of the amide were tolerated. For example, replacing the picolinoyl groups of **5a** with nicotinoyl or isonicotinoyl groups resulted in inactive analogues. The size of the nitrogen-containing heterocyclic group which could be tolerated was limited to monocyclic 4-, 5- or 6-membered rings. Replacing the picolinoyl substituent of **5a** with fused bicyclic moieties such as 2-quinolinecarbonyl or 1-isoquinolinecarbonyl groups resulted in inactive analogues. From these results, it is clear that there is a very stringent requirement for a nitrogen containing heterocyclic, but that the 'nature' of the nitrogen atom can be varied, namely, a secondary amide nitrogen, an aryl sp^2 nitrogen and a secondary amine nitrogen. There is no obvious explanation for the



Scheme 1. (a) Boc-β-Ala, EDC, HOBt, *i*-Pr_2NEt, DMF; (b) TFA, CH_2Cl_2; (c) BocSer(Bn), BOP reagent, HOBt, *i*-Pr_2NEt, DMF; (d) R-CO_2H, BOP reagent, HOBt, *i*-Pr_2NEt, DMF; (e) HF, −78 °C to 0 °C; (f) BocGlu(OBn), BOP reagent, HOBt, *i*-Pr_2NEt, DMF; (g) pGlu, BOP reagent, HOBt, *i*-Pr_2NEt, DMF; (h) Fmoc-*N*(Me)Ser(Bn), BOP reagent, HOBt, *i*-Pr_2NEt, DMF; (i) piperidine; (j) picolinic acid, BOP reagent, HOBt, *i*-Pr_2NEt, DMF.

observed functional group selectivity seen here except to note that this selectivity matches that observed for the peptide SAR around **1**.[12] Also, only the antipode shown of the heterocyclic rings containing stereogenic centers were active (**5b** and **5c**). Combined, these data suggest that there is a very specific interaction of these compounds with the putative receptor.

The Ser units of **5a** were replaced with Thr (D and L), D-Ser, Ala and Gly. None of these substitutions were tolerated at this position. Substituting *N*-methyl-Ser for Ser in **5a** to give **9** was the only variation that was tolerated. This selectivity again mirrors that seen in the pharmacophore region of the peptide **1**, suggesting that these peptidomimetic compounds are targeting the same putative receptor as **1**.

Compound **5a** was chosen for in vivo studies because of its structural similarity to the most potent pharmacophore pattern (picolinoyl-serine) found in the peptide series based on **1**.[12] Compound **1** has been shown to induce HSF from murine bone marrow stromal cells, and that this in turn can enhance effector cell function in mice and afford protection in a murine infection model.[19,20] Like **1**, **5a** enhances effector cell function in mice[21] and is effective in increasing the survival rate of mice challenged with lethal doses of *C. albicans* (Fig. 2).

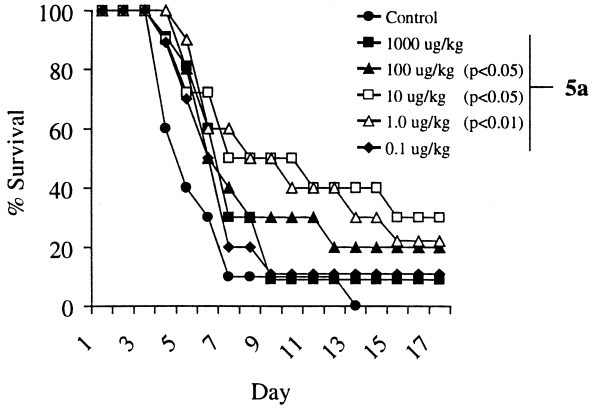


Figure 2. Effect of **5a** on the survival of mice lethally infected with *C. albicans*.

Mice pretreated with 1.0, 10 and 100 μg/kg of **5a** resulted in a statistically significant increase in the survival rate. There were no survivors by day 13 in the diluent treated control group. Similarly, mice pretreated with the lowest dose of **5a** (0.1 μg/kg) failed to demonstrate significant protection. Compound **5a** also demonstrated a 'bell-shaped' dose response that is characteristic of this type of hematoregulatory compound[18] with the 1000 μg/kg dose failing to significantly protect.

Conclusion

We have successfully designed a series of simple, small molecule analogues of a potent hematoregulatory peptide **1**. These compounds retain all of the in vitro biological activity (albeit less potent) of the parent peptide, further validating a pharmacophore model for the interaction of a putative receptor with **1**, **5a**–**e**, **7** and **9**. One possible explanation for the lower potency may be our choice of scaffold. It is possible that the optimal configuration of a structurally simple scaffold has not yet been achieved. Studies to determine the solution phase structure of the scaffold of **1** are underway and may provide further insight. Compound **5a** was further profiled and shown to retain the ex vivo and in vivo biological activity of the parent peptide.

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