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Lysyl 4-aminobenzoic acid derivatives as potent small molecule mimetics of plasminogen kringle 5

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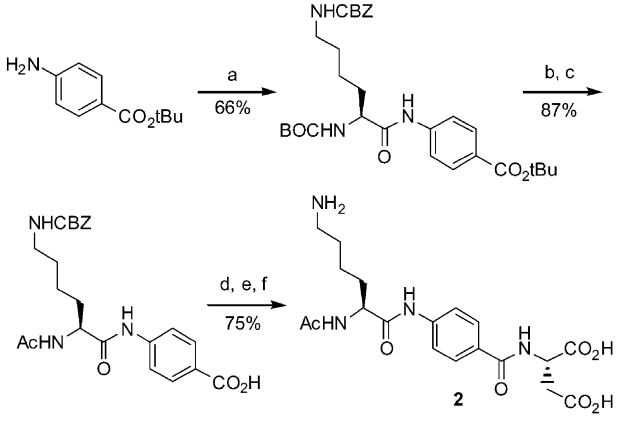
Abstract—Kringle 5, a proteolytic fragment of human plasminogen has been shown to potently inhibit angiogenesis. The tetrapeptide KLYD derived from kringle 5 has been shown to capture many activities of kringle 5 in vitro. Further simplification has been achieved by replacement of the two central amino acids with a 4-aminobenzoic acid spacer group. Molecules displaying the required recognition groups on this core show similar in vitro properties to kringle 5, and are able to displace radiolabeled protein from a high affinity binding site on endothelial cells.

Angiogenesis, the process of formation of new capillaries from existing blood vessels, is a highly regulated physiological process. In normal vasculature, the endothelial cells remain in a quiescent state, and must be activated and induced to undergo a number of processes to create a new, stable structure. This complex process is affected by a number of naturally occurring promoters and inhibitors.[1]

Kringle 5 of plasminogen (K5) has been reported to be a potent inhibitor of angiogenesis and tumor growth.[2] Studies of peptides representing portions of the K5 sequence indicated that the tetrapeptide KLYD found on an inner loop of the kringle structure possessed several key in vitro properties of the protein.[3] In this communication, we report that further structural simplification can be achieved by replacement of the amino acids attached to lysine with appropriately functionalized 4-aminobenzoic acid derivatives.

Studies in search of Ras farnesyltransferase inhibitors had indicated that the activity of CAAX tetrapeptides could be mimicked by replacing the central two hydrophobic amino acids with an aminobenzoic acid spacer.[4] Applying a similar strategy to the KLYD peptide **1**, compound **2** was prepared by the route shown in Scheme 1. *tert*-Butyl 4-aminobenzoate was acylated with a suitably protected lysine derivative activated with di-*tert*-butyl dicarbonate,[5] followed by conversion to the N^{α}-acetyl derivative. Carbodiimide coupling with aspartic acid di-*tert*-butyl ester and deprotection provided **2**. Related compounds were prepared by a similar route and evaluated in an endothelial cell chemotaxis assay to provide an in vitro assessment of anti-angiogenic activity.[6] As indicated in Table 1, **2** was able to inhibit the VEGF-stimulated migration of HMVECs (human microvascular endothelial cells) with a similar potency to K5. Related compounds **3** and **4** with the



Scheme 1. Reagents: (a) BOC-Lys(CBZ)-OH, BOC_2O, pyridine, EtOAc; (b) TFA; (c) Ac_2O, 2,6-lutidine, CH_2Cl_2, THF; (d) H-Asp(O*t*Bu)-O*t*Bu, EDCI, HOBT, NMM, DMF; (e) H_2, Pd/C, MeOH; (f) HCl, dioxane.

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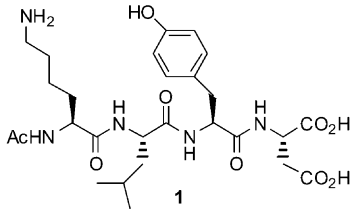
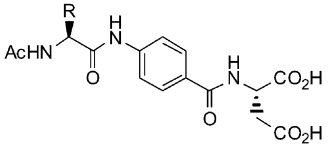
Table 1. Inhibition of VEGF-stimulated migration by K5 and compounds **1**–**4**



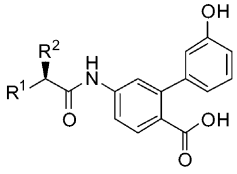
1



Compd	R =	IC_{50}, nM[a]
K5	—	0.015
1	—	1.2
2	$(CH_2)_4NH_2$	0.01
3	H	>100
4	$(CH_2)_4NHAc$	>100

[a] Values are means of three experiments.

Table 2. Inhibition of VEGF-stimulated migration by compounds **5**–**8**

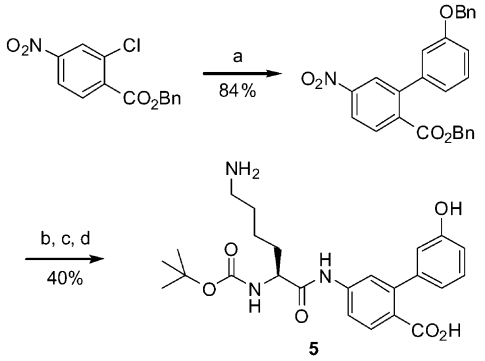


Compd	R^1 =	R^2 =	IC_{50}, nM[a]
5	NHBOC	$(CH_2)_4NH_2$	0.020
6	H	$(CH_2)_4NH_2$	0.06
7	NHBOC	$(CH_2)_3CH_3$	>100
8	NHBOC	$(CH_2)_3NH_2$	1.1

[a] Values are means of three experiments.

lysine sidechain removed or acetylated lost activity in this assay.

While examining the effects of substitution, it was discovered that the aspartic acid residue could be replaced as well. As indicated in Table 2, **5** is a potent inhibitor of HMVEC chemotaxis. As in the aspartate-containing series, the lysine side-chain plays a key role in the activity. Compound **6** lacking N^{α} retains activity, while **7** lacking N^{ε} does not. Compound **8** in which ornithine replaces lysine maintains reasonable activity. Similar results were obtained with other 2-substituted 4-aminobenzoic acids, suggesting that the key interactions are an ammonium and a carboxylate held in the proper geometry. Scheme 2 shows the synthesis of compound **5**; related compounds were prepared in a similar manner.



Scheme 2. Reagents: (a) 3-(BnO)phenylboronic acid, CsF, $Pd(PPh_3)_4$, DME; (b) $SnCl_2$, MeOH, CH_2Cl_2; (c) BOC-Lys(CBZ)-OH, BOC_2O, pyridine, EtOAc; (d) H_2, Pd/C, MeOH.

To confirm that the simplified structures were exerting their effect on HMVEC chemotaxis through the same pathway as K5, a competitive binding study was carried out. K5 has been reported to displace I^{125}-labeled K5 from a high affinity binding site on HMVECs with a K_d of 0.5 nM.[7] Similar studies showed an IC_{50} of 0.7 nM for **5**, supporting the view that it acts as a mimetic of the protein.

In summary, we have demonstrated that small-molecule mimetics of K5 derived from 4-aminobenzoic acid can inhibit VEGF-induced HMVEC chemotaxis, and bind to a high affinity binding site on the target cell with comparable affinity to the protein itself. Further studies will be reported in due course.

References and notes

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6. HMVEC were starved of growth factors overnight, fluorescently labeled with calcein AM (50-100 nM), plated into a migration chamber (2.9×10^4 /well) (Neuroprobe) and stimulated to migrate with VEGF (10 ng/mL). After 4 h, migrated cells were measured by fluorescence. Inhibitors were tested at concentrations from 100–0.001 nM: Majest, S. M.; Bell, R. L. *Proc. Am. Assoc. Cancer Res.* **2001**, *42*, 484.
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