ELSEVIER

On-resin cyclization of peptide ligands of the Vascular Endothelial Growth Factor Receptor 1 by copper(I)-catalyzed 1,3-dipolar azide–alkyne cycloaddition

Victor Goncalves,[a,b] Benoit Gautier,[a,b] Anne Regazzetti,[c] Pascale Coric,[d,e]
Serge Bouaziz,[d,e] Christiane Garbay,[a,b] Michel Vidal[a,b,*] and Nicolas Inguimbert[a,b,*]

[a]*Université Paris Descartes, UFR biomédicale, Laboratoire de Pharmacochimie Moléculaire et Cellulaire,*
45 rue des saints Pères, F-75006 Paris, France
[b]*INSERM U648,45 rue des saints Pères, F-75006 Paris, France*
[c]*Université Paris Descartes, UFR des Sciences Pharmaceutiques et Biologiques, Laboratoire de Chimie Analytique,*
4 avenue de l'observatoire, F-75006 Paris, France
[d]*Université Paris Descartes, UFR des Sciences Pharmaceutiques et Biologiques, Laboratoire de pharmacologie chimique et génétique,*
4 avenue de l'observatoire, F-75006 Paris, France
[e]*INSERM U640, 4 avenue de l'observatoire, F-75006 Paris, France*

Abstract—Cyclic peptides were obtained, on-resin, by the copper (I) catalysed 1,3-dipolar cycloaddition of azides and alkynes. The reaction led exclusively to the formation of the expected cyclomonomeric products which acted as ligands of the Vascular Endothelial Growth Factor receptor 1.
© 2007 Elsevier Ltd. All rights reserved.

In the few years since its discovery, copper (I)-promoted [3+2] Huisgen cycloaddition of azides with terminal alkynes has proved to be one of the most efficient 'click reactions' with widespread applications in organic chemistry and drug discovery.[1] It has been notably used with interest in the field of peptide chemistry to produce 1,2,3-triazoles as peptide bond isosteres[2–4] and to synthesize cyclic peptides. Up to now, two strategies have emerged to perform cyclization of such peptides. The first one was the solution phase method which led to monomeric,[3] dimeric[2] or mixtures of monomeric and dimeric cyclic peptides depending on the nature of amino acids introduced[5] and substrate concentrations.[6] The second strategy was the on-resin cyclization of peptides, as performed by Punna et al.[7] Unexpectedly, this method led to the formation of cyclodimeric products despite the pseudo-dilution effect of the resin.

Herein, we describe the application of copper(I)-catalyzed 1,3-dipolar azide–alkyne cycloaddition to the solid-phase synthesis of cyclic peptides targeting the Vascular Endothelial Growth Factor Receptor 1 (VEGFR1). Interestingly, this method specifically allowed the formation of monomeric peptides.

An increasing number of reports tend to indicate that VEGFR1 is strongly implicated in pathological angiogenesis, a biomarker of cancer, and that its inhibition may constitute an attractive strategy for stopping tumour growth and metastasis.[8–10] VEGFR1 is stimulated by the binding of its pro-angiogenic ligands, the Vascular Endothelial Growth Factor (VEGF) and the Placenta Growth Factor (PlGF). An interesting approach for inhibiting the receptor activation consists in developing peptides, or small molecules, that act as ligands of the receptor extra-cellular domain and therefore are able to displace VEGF/PlGF binding to VEGFR1.[11] Based on the structural[12,13] and mutagenesis[14,15] data available, several amino acids of the VEGF, essentials for VEGF-VEGFR1 interaction were identified (Fig. 1). Considering the spatial proximity existing between some of these
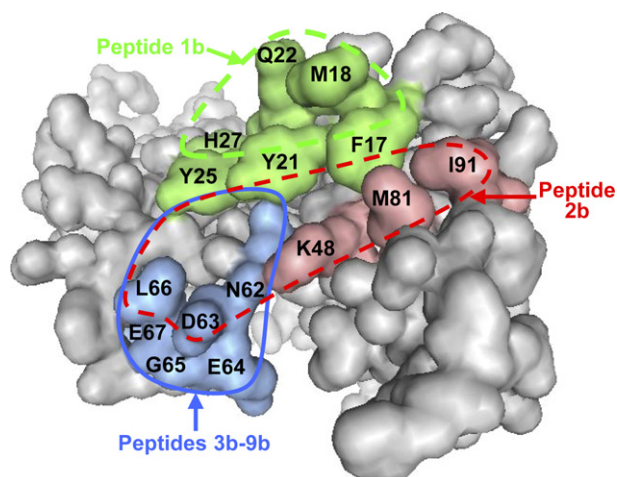
Figure 1. Surface rendering of $VEGF_{165}$ based on the crystal structure of VEGF–VEGFR1$_{d2}$ complex.[12] Residues involved in VEGF–VEGFR1 interaction are colored and labeled. The encircled areas were mimicked by the different synthesized peptides.

residues at the surface of the VEGF, we designed small VEGF mimics as peptides containing these amino acids.

These peptides were cyclised in order to obtain the desired circular arrangements of amino acids. Following this approach, three families of peptides were synthesized (Table 1).

The cyclic peptide **1b** mimics the VEGF residues H27, Q22, M18, F17, Y21, and Y25 (Fig. 1, green line).

The cyclopeptide **2b** mimics L66, D63, N62, K48, M81, I91, F17, W21 and Y25 amino acids (Fig. 1, red line).

A series of peptides (**3b**, **4b**, **5b**, **6b**, **7b**, **8b**, **9b**) imitates the area covered by the VEGF residues Y21, Y25, D63, E64, G65, L66 and E67 (Fig. 1, blue line).

For each cyclic peptide, the corresponding linear product was prepared.

First, the linear peptide **1a** (N_3-GHQMFYY*Pra*-NH$_2$) was synthesized by standard N^{α}-Fmoc chemistry on Rink amide MBHA resin[16] (substitution: 0.62 mmol/g) in 0.25 mmol scale. Couplings were carried out with in situ-activating reagents (HBTU, HOBt in the presence of DIPEA) to generate HOBt esters.

The N-terminal α-azido glycine was synthesized according to the method developed by Lundquist et al.[17] and the alkyne moiety was introduced as a L-propargylglycine introduced in the C-terminal position. This amino acid, commercially available, presents the same stereochemistry as natural amino acids and permits the side-chain cyclization of the peptide. After the elongation completed, a third of the resin was removed, cleaved and deprotected with triisopropylsilane as scavenger, leading to the linear azido-peptide **1a**. The remaining peptidyl-resin was cyclized by exposure to 0.5 equivalents of copper (I) iodide, in presence of sodium ascorbate and 2,6-lutidine in NMP/DCM (Scheme 1, method A). The reaction progress was monitored every 24 h by analyzing a sample of the resin by IR-spectroscopy (disappearance of the 2100 cm^{-1} band characteristic of the azide) and by the modified Kaiser test described by Punna et al.[18]

After 48 h at room temperature, the conversion of the azide and terminal alkyne in 1,2,3-triazole was complete. The β-(1*H*-[1,2,3]triazol-4-yl)alanine amino acid generated from the propargylglycine is abbreviated as βtA in the following cyclopeptides. The peptide was then cleaved from the resin, deprotected and purified by HPLC to a purity of 95%. After these treatments, the cyclomonomeric peptide **1b** (cyclo[GHQMFYYβtA]-NH$_2$) was exclusively isolated. The linear and cyclic peptides **2**–**6** were synthesized according to the same

Table 1.

Compound	Peptide sequence[a]	Activity at 100 μM (%)[b]	IC50 (μM)
1a	N_3-GHQMFYYPra-NH$_2$	67	19.4 ± 2.2
1b	Cyclo[GHQMFYYβtA]-NH$_2$	35	121 ± 29
2a	N_3-GLDNKMIFWYPra-NH$_2$	95	22.9 ± 4.2
2b	Cyclo[GLDNKMIFWYβtA]-NH$_2$	83	31.1 ± 1.2
3a	N_3-GhFDEGLEPra-NH$_2$	0	ND
3b	Cyclo[GhFDEGLEβtA]-NH$_2$	10	ND
4a	N_3-GhFDEPLEPra-NH$_2$	0	ND
4b	Cyclo[GhFDEPLEβtA]-NH$_2$	29	ND
5a	N_3-YYDEPLEPra-NH$_2$	0	ND
5b	Cyclo[YYDEPLEPra]-NH$_2$	0	ND
6a	N_3-FYDEPLEPra-NH$_2$	55	96 ± 14
6b	Cyclo[FYDEPLEβtA]-NH$_2$	61	93 ± 18
7a	N_3-QYDEPLEPra-NH$_2$	0	ND
7b	Cyclo[QYDEPLEβtA]-NH$_2$	0	ND
8a	N_3-EYDEPLEPra-NH$_2$	6	ND
8b	Cyclo[EYDEPLEβtA]-NH$_2$	7	ND
9a	N_3-KYDEPLEPra-NH$_2$	27	151 ± 29
9b	Cyclo[KYDEPLEβtA]-NH$_2$	28	162 ± 24
SP5.2	NGYEIEWYSWVTHGMY-NH$_2$	99	28 ± 7

ND, not determined; In this set of experiments, recombinant human $VEGF_{165}$ displayed an IC_{50} of 387 ± 60 pM.
[a] hF, homophenylalanine; Pra, propargylglycine; βtA, β-(1*H*-[1,2,3]triazol-4-yl)alanine.
[b] The inhibitory activity corresponds to the percentage of biotinylated $VEGF_{165}$ displaced by 100 μM of peptide on VEGFR1.