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Design, synthesis and in vitro biological evaluation of a small cyclic peptide as inhibitor of vascular endothelial growth factor binding to neuropilin-1

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ABSTRACT

Neuropilin-1 (NRP-1) is a co-receptor of VEGFR (vascular endothelial growth factor receptor), but it is also suggested that NRP-1 in tumour cells may serve as a separate receptor for $VEGF_{165}$. Therefore molecules interfering with $VEGF_{165}$ binding to NRP-1 seem to be promising candidates as new anti-angiogenic and anti-tumour drugs. Here, we report the design, synthesis, biological evaluation and molecular modelling of the small cyclic peptide, which shows a good inhibitory effect on $VEGF_{165}$/NRP-1 binding (IC_{50} = 0.18 μM). The reported compound could be considered as one of the smallest cyclic peptides (MW = 510) interfering with $VEGF_{165}$/NRP-1 binding presented up to now.

Angiogenesis, the physiological process of new blood vessels formation, was first suggested as an essential component of tumour progression by Folkman in 1971.[1] Tumour growth relies on the development of new vasculature that delivers oxygen and nutrients to proliferating cells and allows simultaneous removal of carbon dioxide as well as metabolic waste. Additionally, it provides a route for spreading of malignant cells over the body. Therefore the inhibition of angiogenesis is one of the most promising approaches to treat cancer.[2] The body controls angiogenesis by producing a precise balance of growth and inhibitory factors in healthy tissues. The fundamental pro-angiogenic signalling molecule is vascular endothelial growth factor (VEGF), which promotes proliferation, survival, migration and permeability of endothelial cells lining the inner layer of blood vessels.[3] $VEGF_{165}$, the dominant isoform of VEGF glycoprotein, is responsible for pathological angiogenesis.[4,5] In endothelium, the pro-angiogenic function of $VEGF_{165}$ is mediated via VEGFR-2, also known as kinase insert domain-containing receptor (KDR).[6] VEGF binding to its receptor is associated with the receptor dimerization and activation that triggers downstream signalling resulting in stimulation of cell proliferation. Other responses related to VEGF, including cell migrations, blood vessel guidance and branching, involve a more complex receptor assembly containing VEGFR-2 co-receptor neuropilin 1 (NRP-1).[7,8]

NRP-1 is found primarily in arterial endothelial cells, but its presence has been also demonstrated in cancer cell lines as well in various primary tumours, e.g., breast,[9–12] lung,[13–15] skin,[16,17] glioma,[18–20] colon,[21] gastric[22] and prostate[23] cancers. Recently, much evidence coming from different groups has suggested that NRP-1 may serve in tumour cells as a separate receptor for VEGF.[24] These data triggered interest in searching for new molecules interacting with NPR-1 that would inhibit pathological angiogenesis and could become prospective anti-tumour drugs.[25,26] Current efforts have focused on the development of anti-NRP-1 monoclonal antibodies blocking interaction with $VEGF_{165}$ or on identification of specific peptide or small molecule inhibitors of $VEGF_{165}$/NPR-1 binding.[27] The search for peptide inhibitors have identified different specific linear and even bicyclic peptides. Jia and cowork-

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; BSA, bovine serum albumin; (bt)-$VEGF_{165}$, biotinylated-$VEGF_{165}$; 6-Cl·HOBt, 6-chloro-1-hydroxybenzotriazole dehydrate; DIPEA, *N*,*N*-diisopropylethylamine; ELISA, enzyme-linked immunosorbent assay; HUVEC, Human Umbilical Vein Endothelial Cells; MDA-MB-231, human breast adenocarcinoma cells; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium salt; PBS, phosphate buffered saline; SPPS, solid phase peptide synthesis; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyl-uroniumtetrafluoroborate; VEGF, vascular endothelial growth factor.

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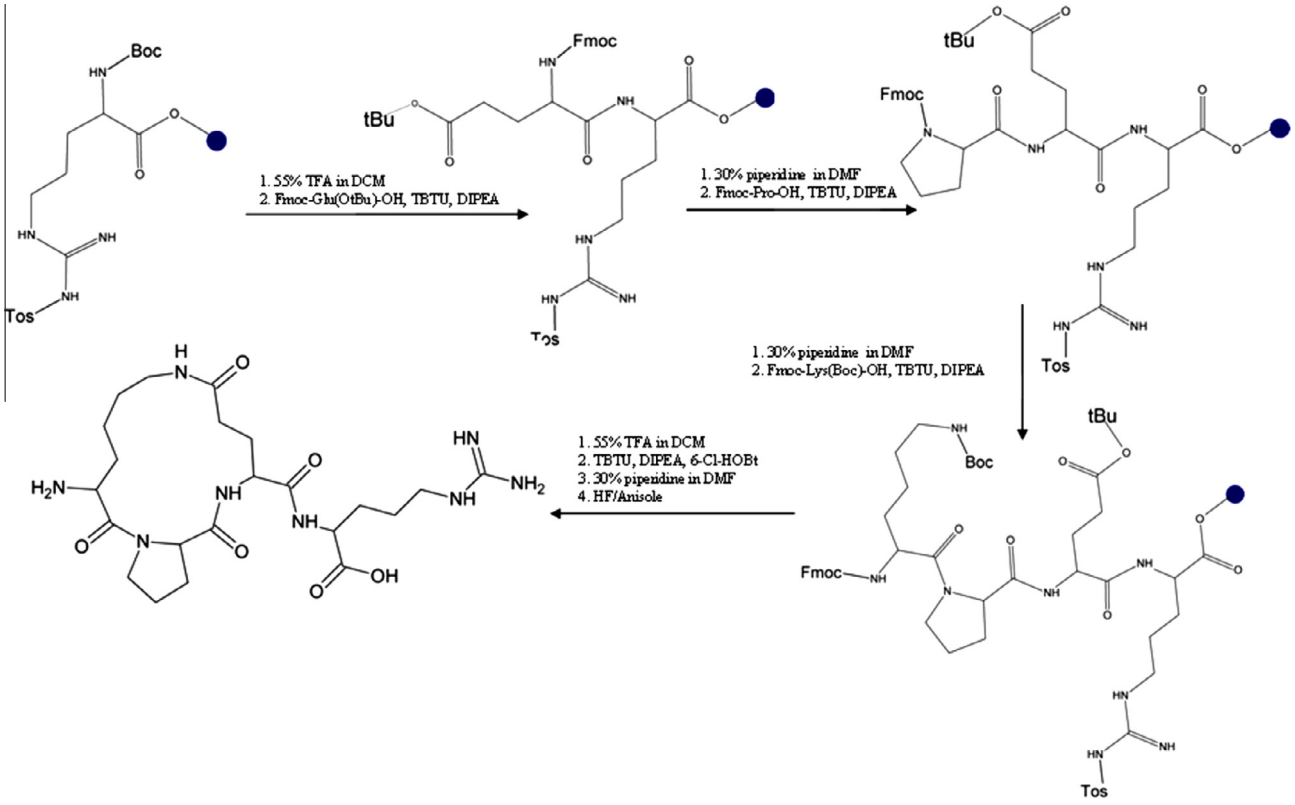

Scheme 1. The synthesis of H-c[Lys-Pro-Glu]-Arg-OH.

Table 1
Inhibitory effects and IC_{50} of A7R and H-c[Lys-Pro-Glu]-Arg-OH

Peptide	% inhibition of $VEGF_{165}$ binding to NRP-1 in a different concentration							IC_{50} (μM)
	100 μM	10 μM	3 μM	1 μM	0.3 μM	0.1 μM	0.01 μM	
A7R	87.8 ± 2.6	61.0 ± 0.4	38.1 ± 3.7	24.4 ± 3.5	14.6 ± 2.3	2.9 ± 2.1	NS	5.86
H-c[Lys-Pro-Glu]-Arg-OH	100 ± 3.1	94.7 ± 0.7	86.7 ± 1.3	76.5 ± 0.5	61.7 ± 0.5	35.5 ± 4.1	3.1 ± 8.3	0.18

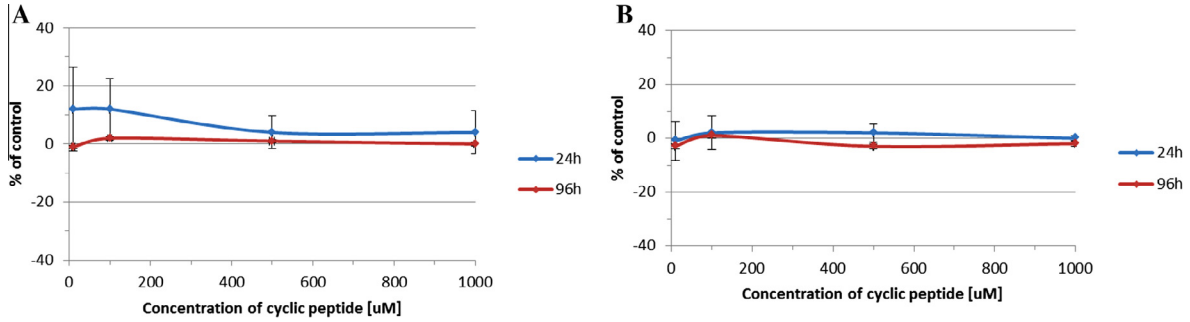

Figure 1. Influence of H-c[Lys-Pro-Glu]-Arg-OHon (A) HUVEC and (B) MDA-MB-231 cells viability after 24 and 96 h incubation.

ers demonstrated that peptides encoded by VEGF exon 6 inhibit VEGF biological effects.[28] Further studies led to developing of specific bicyclic peptides based on C-terminal amino acids of $VEGF_{165}$.[29,30] Another specific peptide was identified by screening a mutated phage library by Starzec et al.[31] The authors isolated a heptapeptide ATWLPPR (A7R) which exhibits in vitro and also in vivo anti-angiogenic activity.[31] It was found that in vivo treatment with A7R resulted in decreasing angiogenesis and tumour growth in breast cancer cell xenograft mice. Structure–activity relationship study of A7R has shown that C-terminal arginine is crucial for interaction with NRP-1 and the shortest fragment of this peptide which retains activity is LPPR.[32]

Application of synthetic therapeutic peptides as prospective drugs is recently growing since peptides have many advantages, e.g., high potency and selectivity and less toxicity compared to small organic compounds.[33] Disadvantages of using peptides as drugs is their short half-life and fast elimination from human plasma. To reduce or at least minimize these weaknesses without decreasing biological effects different modifications of peptide molecules were proposed. One of the more often used methods