Inhibitors of the Neuropilin-1 and Vascular Endothelial Growth Factor 165 interaction

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Introduction

Neuropilin-1 (NRP-1) is a protein expressed by a number of types of somatic cells [1]. Moreover, it has been found to be overexpressed in several kinds of malignant tumors e.g. breast cancer [2] and it is postulated that in pathological angiogenesis its interaction with the Vascular Endothelial Growth Factor 165 (VEGF₁₆₅) leads to progression of tumor vascularization and growth [3]. Additionally, this signaling complex is also involved in suppression of immune response against tumor cells [4].

Figure 1: Scheme of signaling pathways mediated by NRP-1/VEGF₁₆₅ involved in tumor development and its escape from immune surveillance

Results and Discussion

Inhibitors of the NRP-1/VEGF₁₆₅ complex are very attractive compounds to block several processes regulating tumor growth and metastasis. In the frame of presented project efforts have been made to design strong inhibitors of this interaction. All compounds were synthesized and characterized with HPLC and mass spectrometry. By the means of ELISA-based test and molecular dynamics simulations, the inhibitory activity toward NRP-1/VEGF₁₆₅ complex has been characterized. Lead compounds were tested in an preliminary assay toward proteolytic stability in human serum.

The presented paper is a review of our recent four publications on peptidic or peptidomimetic inhibitors of NRP-1/VEGF₁₆₅ complex.

Conformational latitude – inhibitory activity relationship of KPPR tetrapeptide analogues (KxxR)[5]

Several analogues (KxxR) with various conformational latitudes have been synthesized and found as inhibitors of NRP-1. Detailed insight provided by molecular dynamics simulation allowed forming a clear relationship between “flexibility” of the -xx- part (positions 2 and 3) of the molecules and their inhibitory activity. Introduction of amino acids with greater conformational latitude decreases inhibitory activity in a non-equal manner. The most important sites of the molecule are N-terminal K and C-terminal R, which directly interact with the NRP-1. The -xx- part allows the remaining part of the molecule to find interaction partners on the NRP-1 surface and by this greatly affects the inhibitory activity. For sufficient inhibitory activity, proline with low conformational latitude in position 3 should be maintained (KxPR).

Structure – inhibitory activity relationship study of tetrapeptide inhibitors [6]

Here we present a structure–activity relationship study of the systematic optimization of amino acid residues in positions 1–3 (Kxx). Substitution of N-terminal K in position 1 to homoarginine (Har) increases the inhibitory activity. The HarPPR analogue has been used as the next starting compound for further modifications. Position 2 is the best site to incorporate basic amino acid such as Dap or Dab. Analogue with Dab is the strongest NRP-1 inhibitor with sub-micromolar IC_{50} ($0.8 \mu M$) in this generation.

Branched pentapeptides and cyclic peptides as potent inhibitors [7], [8]

Our findings suggest that branching at position 1 with additional homoarginine (Har) residue, to obtain K(Har)PPR, allows more effective inhibition. Increasing the conformational latitude of the middle part of molecule, in particular with simultaneous introduction of basic amino acids at the second or third position, produced lead compounds K(Har)DabPR and K(Har)PDabR (both with IC_{50} $0.2 \mu M$). These two linear and branched pentapeptides exhibit the same inhibitory activity, however the first one is more stable toward proteolysis in human serum (half-lives are 41 h and 44 min).

Moreover, simple side chain – side chain cyclization of EPKR peptide resulted in obtaining a very potent inhibitor c[EPK]R (A) with $IC_{50} = 0.16 \mu M$. Also dimeric forms of such compound possess inhibitory activity (B) $IC_{50} = 0.89 \mu M$

Figure 2: Structures of two exemplary cyclic inhibitors of NRP-1/VEGF165 complex

In the recent years novel peptide therapeutics have been approved for clinical use. Therefore the field of peptide based inhibitors of NRP-1 could be a promising direction for development of new antitumor drugs.

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References

- [1]. F. Nakamura and Y. Goshima, Neuropilin: From Nervous System to Vascular and Tumor Biology, D. Bagnard, Ed. Boston, MA: Springer US, pp. 55–69 (2002).
- [2]. A. Jubb M et al., J. Pathol., vol. 226, no. 1, pp. 50–60, (2011).
- [3]. H. L. Goel and A. M. Mercurio, Nat. Rev. Cancer, vol. 13, p. 871, (2013).
- [4]. M. Terme et. al., Oncoimmunology vol. 2, no. 8, (2013).
- [5]. B. Fedorczyk et al., J. Pept. Sci., vol. 23, no. 6, pp. 445–454, (2017).
- [6]. D. Tymecka et al., Peptides, vol. 94, pp. 25–32, (2017).
- [7]. D. Tymecka et al., Eur. J. Med. Chem., vol. 158, pp. 453–462, (2018).
- [8]. K. Grabowska et. al., Bioorg. Med. Chem., vol. 25(2), pp. 597–602 (2017).