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Carbohydrate-based peptidomimetics targeting neuropilin-1: Synthesis, molecular docking study and in vitro biological activities

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ABSTRACT

Neuropilin-1 (NRP-1), a transmembrane glycoprotein acting as a co-receptor of VEGF-A, is expressed by cancer and angiogenic endothelial cells and is involved in the angiogenesis process. Taking advantage of functionalities and stereodiversities of sugar derivatives, the design and the synthesis of carbohydrate based peptidomimetics are here described. One of these compounds (**56**) demonstrated inhibition of VEGF-A_{165} binding to NRP-1 (IC_{50} = 39 μM) and specificity for NRP-1 over VEGF-R2. Biological evaluations were performed on human umbilical vein endothelial cells (HUVECs) through activation of downstream proteins (AKT and ERK phosphorylation), viability/proliferation assays and in vitro measurements of anti-angiogenic abilities.

1. Introduction

Angiogenesis or formation of new blood vessels requires the binding of signaling molecules, such as vascular endothelial growth factor (VEGF), which is one of the most specific and important growth factors involved in this process.[1,2] VEGF-A_{165} mediates its biological effects through receptors located on the endothelial cells, i.e., VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR). VEGF-A_{165} is overexpressed by a wide variety of human tumors and this overexpression has been correlated with invasion and metastasis.[3]

Interestingly, Neuropilin-1 (NRP-1), a receptor protein firstly described in neuronal guidance,[4] is involved in a wide range of physiological and pathological processes including angiogenesis.[5] This transmembrane protein was found to be a co-receptor of VEGF-A_{165} acting together with VEGFR-2 via its NRP-1 b1/b2 domain, resulting in increased affinity of VEGF-A_{165} for the extracellular domain of VEGFR-2.[6] VEGF-A_{165} can also contribute to VEGFR-2/NRP-1 complex formation via its binding through two different sites for both NRP-1 and VEGFR-2.[7] Moreover, independently of VEGFR-2, NRP-1 alone might transduce functional signaling as a result of VEGF binding.[8] NRP-1 is expressed on endothelial cells promoting tumor angiogenesis[6,9] and on tumor cells and has thus been identified as a potential target for anti-angiogenic therapies.[10,11] This receptor could also be a mediator of choice for cancer imaging. Few attempts to use NRP-1 in cancer imaging via a [99mTc]-labeled peptide,[12] multifunctional nanoparticles engineered with gadolinium chelates as MRI contrast agents,[13] and fluorescein-labeled peptides are reported in the literature.[14] More recently, NRP-1 targeting peptides were conjugated onto the surface of lipid microbubbles for molecular imaging of tumor angiogenesis.[15]

Targeting NRP-1 with small molecules mimicking VEGF-A_{165} is not very advanced, because of difficulty to mimic protein–protein

Abbreviations: MRI, magnetic resonance imaging; AKT, serine/threonine protein kinase; ERK1/2, extracellular signal-regulated protein kinases 1 and 2.

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interactions. Nevertheless, several peptides have been reported to modulate VEGF-A_{165}/NRP-1 binding. The first crystal structure of the b1 domain of the human NRP-1 was determined by Lee et al.[16] The role of the loops at b1 domain of human NRP-1 as a target binding site for ligand interaction has also been highlighted by Vander Kooi et al. with their study of NRP-1 binding with tuftsin (TKPR), which is very similar to the VEGF-A_{165} C-terminus (DKPRR).[17] The C-terminal arginine of tuftsin contributes to the majority of interactions with NRP-1 and this was confirmed by a molecular modeling approach developed by Haspel et al.[18] The interaction of NRP-1-b1 domain with VEGF-A_{165} was recently elucidated by Parker et al.[19] Jia et al. have discovered a specific bicyclic peptide EG3287 antagonist of VEGF-A_{165} binding to NRP-1.[20] Heptapeptide ATWLPPR (A7R), selected by screening a phage display library was described as an effective antagonist and can be considered as a potent inhibitor of tumor angiogenesis.[21] NRP-1 targeting photodynamic therapy (PDT) using A7R as NRP-1 ligand associated with a porphyrin-like sensitizer has since been developed.[22] New peptides structurally related to VEGF-A_{165} exon-7 and -8 domains were recently designed and synthesized.[23]

However, peptidic compounds could suffer from poor bioavailability and instability[24] and the design of new organic molecules such as peptidomimetic derivatives seems an attractive alternative. In connection with our ongoing program on the design of sugar-based peptidomimetics, we described a few years ago the design, synthesis and in vitro biological evaluation of sugar-based peptidomimetics targeting NRP-1. Interesting compounds were obtained and one hit compound was identified (compound **1**, Chart 1a).[25] Simultaneously, a paper by Jarvis et al. described compound EG00229 as small molecule inhibitor of the NRP-1/VEGF-A_{165} binding and this molecule has recently shown in vivo activity towards cancer models (Chart 1a).[26] More recently, a fully non-peptidic compound comprising phenylbenzimidazole and benzodioxane moieties linked by a stable carboxythiourea spacer was identified.[27] New antagonists structurally-related to this compound were synthesized and evaluated toward VEGF-A_{165}/NRP-1 binding modulation.[28] Several drug-like compounds containing a common chlorobenzyloxy alkyloxy halogenobenzyl amine scaffold were identified by virtual screening for their efficient binding to NRP-1.[29]

The objective of the present work was the molecular design and synthesis of new NRP-1 ligands inspired by compound **1**.[25] The use of carbohydrates as scaffolds to construct bioactive compounds and peptidomimetics is a well-accepted concept since the pioneering works related to peptidomimetics of somatostatin.[30–32] We wanted to take advantage of functionalities and stereodiversities of sugar derivatives to develop new analogs of carbohydrate-based peptidomimetics. Thus, sugar scaffolds functionalizable at both ends and offering different spatial orientations for residues were investigated according to molecular modeling and the in vitro biological properties of the new ligands were evaluated.

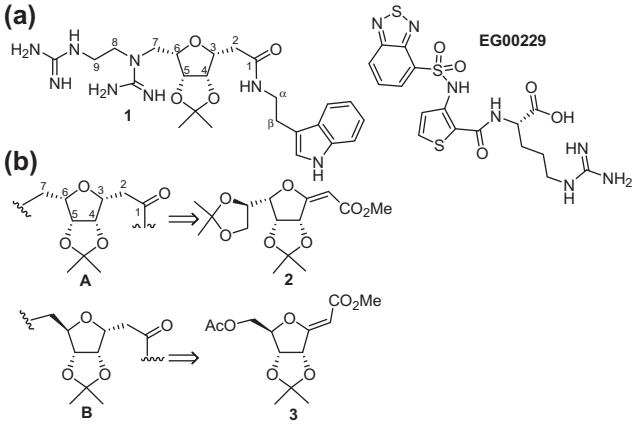

Chart 1. (a) Structure of compound **1** and EG00229. (b) Structure of scaffolds A and B functionalizable at both ends and structure of starting *exo*-glycals **2** and **3**.

2. Results

Sugar scaffolds A and B suitable for functionalization at both ends could arrange crucial residues in opposite spatial directions and were both investigated in this regard (Chart 1b). Firstly, compound **1** was modified stepwise, the importance of the two guanidine functions was studied and the effect of hydrocarbon linker lengths separating the guanidinium groups was explored (first series). A second series with an arginine anchored at C1 was developed according to docking study. In the meantime, synthetic compounds were used to get crystals of NRP-1/ligand complex. The binding affinity of new derivatives was performed by ELISA and the effects of the most potent ligands were evaluated on HUVECs through activation of downstream proteins, viability assays and in vitro angiogenic abilities.

2.1. Synthetic chemistry

The *C*-glycosidic scaffold A which was used for compound **1** synthesis was obtained from the known *exo*-glycal **2** available in excellent yield via a Wittig reaction on the D-gulono-1,4-lactone.[33] In the same way, scaffold B was obtained from *exo*-glycal **3** directly available from the corresponding D-ribono-1,4-lactone. The first series aimed at synthetizing new peptidomimetic compounds close to compound **1** structure. The impact of the spatial disposition of both guanidinium groups and 2-(indolyl-3-yl)ethyl residue was evaluated by synthesizing a compound based on the sugar scaffold B. Moreover, while maintaining compound **1** central core (scaffold A) with the 2-(indol-3-yl)ethyl residue at C1, the effect of the linker length between both guanidinium groups was considered. The replacement of one of the guanidinium functions by a triazole moiety acting as a stable linker[34–36] was envisioned.

The synthetic pathway for the first series required the preparation of the key tosylates **8** and **24** (Scheme 1A and B). The known *exo*-glycal **2** was converted to *C*-glycoside **4** by stereoselective reduction of the double bond.[37] Subsequent saponification of **4** followed by coupling with tryptamine led to amide **6**. Alcohol **7** was prepared by a multistep sequence including the selective removal of the exocyclic isopropylidene acetal under acidic conditions, $NaIO_4$ mediated oxidative cleavage and reduction of the resulting aldehyde. The alcohol **7** was then converted in the corresponding tosylate **8** in excellent yield. In the same way, *exo*-glycal **3** was converted in *C*-glycoside **18**. After changing acetate protecting group at O7 for a *tert*-butyldimethylsilyl ether, subsequent saponification of **20** led to the key acid **21** in good yield. Amide **22** was prepared by coupling with tryptamine. Removal of the *tert*-butyldimethylsilyl group was achieved by TBAF and the resulting alcohol was converted in tosylate **24** in 87% yield.

Refluxing tosylates **8** and **24** with different commercially available monoprotected diaminoalkanes ($n = 2$, 3 and 4 for **8** and $n = 1$ for **24**, Scheme 1C) in a sealed flask led to intermediates **9**, **10**, **11** and **25**. Free amines obtained in quantitative yields after removal of benzyloxycarbonyl group were treated with *N*,*N*′-bis(benzyloxycarbonyl)-*S*-methylisothiourea[38] in DMF. Bis-guanidinylated derivatives **12**, **13**, **14** and **26** were obtained in modest yields, resulting from the reaction of both primary and secondary amines. Finally, removal of protecting groups afforded the expected bis-guanidinoglycosides **15**, **16**, **17** and **27**. Attempts to remove the 4,5-isopropylidene group under different acidic conditions were