Drug Selectivity

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Increasing αvβ3 Selectivity of the Anti-Angiogenic Drug Cilengitide by N-Methylation**

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The drug Cilengitide, *c*(RGDf(*N*Me)V), is a cyclic RGD pentapeptide (R = arginine, D = aspartic acid, G = glycine) currently in clinical phase III for the treatment of brain tumors and in phase II for other cancer types.[1] The antitumoral properties of this peptide are based on its antagonistic activity for pro-angiogenic integrins, such as αvβ3, αvβ5, or α5β1. However, the specific roles of these integrin subtypes in angiogenesis and cancer are not yet clear and fully understood. In this work, we present di-N-methylated analogues of the stem peptide *c*(RGDfV) which retain an αvβ3-binding activity in the nanomolar range but have lost most of the activity for integrins αvβ5 and/or α5β1. Highly active and selective peptides for αvβ3 are important tools to study the specific role of this integrin in angiogenesis and cancer.

Integrins are heterodimeric receptors that govern cell–cell and cell–extracellular matrix (ECM) interactions, and play crucial roles in a plethora of cellular functions.[2] The fact that many integrins are involved in pathological processes, such as tumor angiogenesis, has stimulated their study as therapeutic targets.[3] A number of integrin receptors recognize and bind the tripeptide sequence RGD, which is a prominent cell-adhesion motif present in ECM proteins.[4] Mimicking this tripeptide sequence with RGD-peptides or peptidomimetics is hence a promising approach to target integrins involved in angiogenesis and to develop anti-cancer agents.[1,3b,5]

It is known that αvβ3 and αvβ5 are involved in two different angiogenic pathways.[6] Whereas angiogenesis induced by basic fibroblast growth factor (bFGF) or tumor necrosis factor-α depends on αvβ3, angiogenesis triggered by vascular endothelial growth factor (VEGF) or transforming growth factor-α is αvβ5-dependent. These two integrins are also described to be important mediators in the regulation of hypoxia in glioblastomas.[7] However, mice lacking either αv or β3 and β5 integrins showed extensive angiogenesis.[8] These intriguing results were a matter of debate and challenged our understanding about the role of these two integrins in angiogenesis.[9] The integrin α5β1 is also highly expressed in angiogenic vasculature by several angiogenic stimuli, such as bFGF but not by VEGF.[10] Since αvβ3, αvβ5 and α5β1 have partially overlapping ligand affinities,[4b] it is plausible that α5β1 might substitute the pro-angiogenic activity of the other integrins. Paradoxically, another recent study showed that low concentrations of Cilengitide stimulates VEGF-mediated angiogenesis.[11] Although the doses used in this study are far lower than therapeutic concentrations[12] and hence such a "pro-angiogenic" effect is not likely to be observed in the clinical studies, it becomes evident that a better understanding of anti-angiogenic agents is necessary.[13]

It has been shown by us and others that N-methylation can increase the selectivity towards specific receptor subtypes.[14] These biological effects are often caused by the induction of conformational constraints in the peptide backbone, which lead to preferred single conformers essential for biological activity.[14a,d,h,15] Thus, we envisioned that further N-methylation of Cilengitide could result in enhanced selectivity profiles. For this reason we designed a library containing all the di-N-methylated analogues of *c*(RGDfV) (Figure 1).

Note that the synthesis of *N*Me peptides (especially if they are cyclic) is not without challenges that need to be carefully considered.[14a,16] In the first place, although many N-methyl amino acids are commercially available, most of them are still expensive. Therefore, we synthesized, in solution, the *N*Me residues of Gly, Val, and D-Phe by reduction of the corresponding oxazolidinone using Freidinger conditions.[17] Alternatively, Arg and Asp were methylated on resin using the Miller and Scanlan method,[18] later optimized by Biron et al.,[19] which is compatible with acid-sensitive side-chain

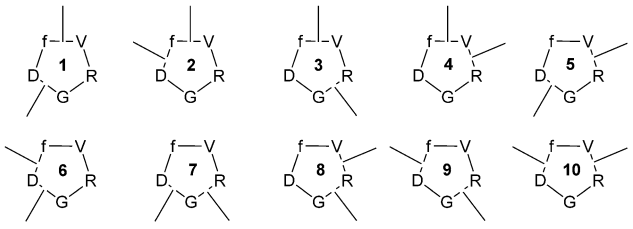

Figure 1. Schematic representation of our library of di-N-methylated analogues of *c*(RGDfV).

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