Tumor Targeting via Integrin Ligands: Synthesis and Biological Evaluation of RGD Peptidomimetic-Paclitaxel Conjugates

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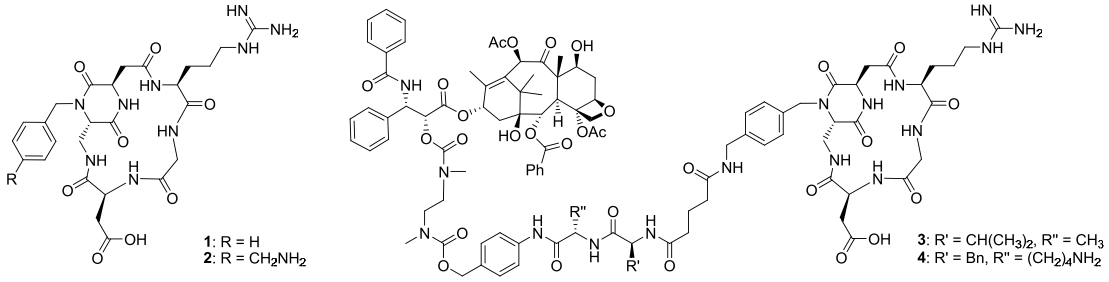
Integrins are a large family of heterodimeric transmembrane glycoprotein receptors, composed by two non-covalently-associated subunits (α and ß). Integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$ have been found to be overexpressed on blood vessels in human tumors, but not on vessels in normal human tissues. For this reason, these integrins have become attractive targets for pharmacological studies mainly in the oncology area. The Gennari and Piarulli group recently developed a peptidomimetic compound (**1**) containing the RGD (Arg-Gly-Asp) sequence and a diketopiperazine (DKP) scaffold as powerful $\alpha_V\beta_3$ integrin ligand.[1] A functionalized analogue of this ligand (**2**)[2] was linked to Paclitaxel through two lysosomally cleavable linkers (namely the Val-Ala and Phe-Lys peptide sequences).[3]



	IC_{50} (nM)		
Structure	CCRF-CEM ($\alpha_v\beta_3$ −)	CCRF-CEM $\alpha_v\beta_3$ ($\alpha_v\beta_3$ +)	Selectivity
Paclitaxel	155 ± 55	21 ± 2	7.4
RGD-Val-Ala-PTX (**3**)	5153 ± 977	77 ± 20	66.9
RGD-Phe-Lys-PTX (**4**)	535 ± 70	34 ± 2	15.7

Table 1: Antiproliferative activity of *cyclo*[DKP-RGD] conjugates in CCRF-CEM and CCRF-CEM $\alpha_v\beta_3$ after 6 hour-treatment followed by compound washout and 138 hour-long growth in fresh medium.

The resulting compounds **3** and **4** were subjected to stability assays in the presence of cathepsin B and lysosome extract, revealing that the free Paclitaxel is efficiently released under these conditions. The antiproliferative activities of the conjugates were evaluated against two isogenic cell lines expressing $\alpha_V\beta_3$ at different levels: the acute lymphoblastic leukemia cell line CCRF-CEM ($\alpha_V\beta_3$ −) and its subclone CCRF-CEM $\alpha_V\beta_3$ ($\alpha_V\beta_3$ +). A fairly effective integrin-targeting was displayed by conjugate **3**, which was found to inhibit cell proliferation with increased selectivity towards $\alpha_V\beta_3$-expressing cells compared to the free PTX (Table 1).

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[2] Colombo, R.; Mingozzi, M.; Belvisi, L.; Arosio, D.; Piarulli, U.; Carenini, N.; Perego, P.; Zaffaroni, N.; De Cesare, M.; Castiglioni, V.; Scanziani, E.; Gennari, C. *J. Med. Chem.* **2012**, *55*, 10460.
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