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Synthesis of enantiomerically pure $\alpha_v\beta_3$ integrin ligands based on a 5,6-dihydropyridin-2-one scaffold

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Abstract—The fundamental importance of $\alpha_v\beta_3$ integrin in a diverse range of biological processes, makes the search for new ligands of this receptors a significant therapeutic goal. We herein report our initial results on the synthesis of 5,6-dihydropyridin-2-one based ligands, containing a rigid heterocyclic core and two appendages mimicking arginine and aspartic acid moieties. In particular, we explored the influence of the scaffold stereochemistry on bioactivity, performing SK-MEL-24 cell-fibronectin adhesion tests on diastereoisomers, that differ in the configuration at C6.

1. Introduction

Many functions in multicellular organisms involve complex interactions between cells and the extracellular matrix (ECM).[1] Integrin-mediated cell adhesion is therefore of fundamental importance in a diverse range of biological processes, including cell differentiation, embryonic cell migration, maintenance of tissue integrity and blood coagulation.[2]

In the family of integrin, the $\alpha_v\beta_3$ is one of the more versatile receptors since it binds through the tripeptide sequence RGD (Arg-Gly-Asp) to a number of ECM components such as fibronectin, fibrinogen, vitronectin and osteopontin.[3] This integrin is expressed on several malignant tumour infiltrating endothelial cells and represents an attractive investigational target for therapeutic intervention.[4] In addition, the preferential expression of $\alpha_v\beta_3$ integrin on new forming blood vessels enhances its potential application as an antitumour target, since tumour proliferation and metastasis are highly dependant on neovascularization.[5] The $\alpha_v\beta_3$ receptor is also highly expressed in osteoclasts, the cells responsible for bone resorption, and it is thought to be involved both in cellular adhesion of osteoclasts and in regulating their migration along the bone surface.[6] For these reasons, the reduction of osteoclast activity by antagonism of integrin $\alpha_v\beta_3$, offers a potential therapy for the prevention and treatment of osteoporosis.[7]

Linear and cyclic peptides,[8] containing the RGD sequence, have been reported as excellent ligands of integrin $\alpha_v\beta_3$ and have significant therapeutic potential but serious limitations, especially for oral dosing. The need for antagonists with higher bioavailability and lower molecular weight has prompted several research groups to develop small constrained non-peptidic molecules mimicking the RGD motif, which would be more promising for drug development.[9] Most of the structures proposed so far, share a common pattern, consisting of a polyfunctionalized rigid core, linked to appendages corresponding to arginine and aspartic acid side chains. The basicity and length of the arginine-mimicking group was found to play a central role.[10] Moreover, the presence of a carboxylic function, mimicking the aspartic acid residue in the original binding motif, is a fundamental feature to create a ionic interaction with the metal cation in the receptor active site.[11]

We envisaged 3-bromo-5,6-dihydropyridin-2-one[12] as a scaffold that could be converted into a potential $\alpha_v\beta_3$ integrin ligand introducing a carboxylic acid and a guanidinic appendage (Fig. 1). Based on these premises, we embarked on synthetic efforts to explore whether this

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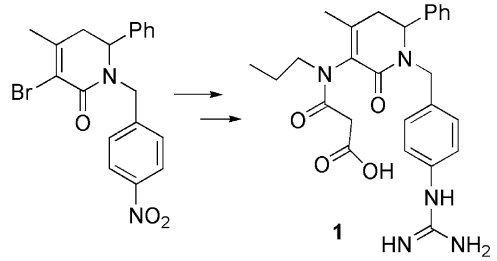


Figure 1. Racemic peptidomimetic **1**, containing 5,6-dihydropyridin-2-one as a rigid core.

scaffold could adopt a rigid conformation, amenable for the interaction with the integrin binding site and we synthesized racemic compound **1**.[13]

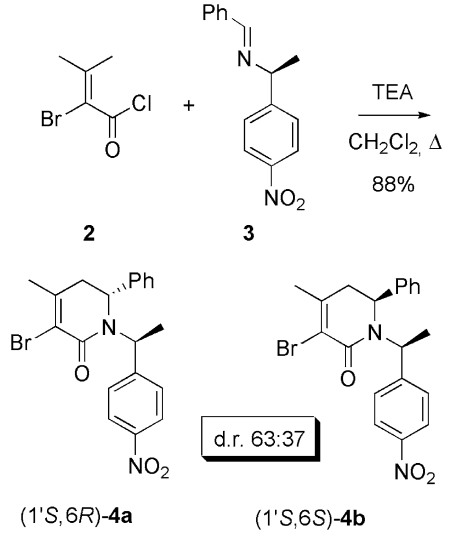
2. Results and discussion

The activity and selectivity of **1** on RGD recognizing integrins in a cellular environment was evaluated performing cell adhesion assays both on melanoma cell line SK-MEL-24 ($\alpha_v\beta_3^{POS}$)[14] and human erythroleukemic cell line K562 ($\alpha_5\beta_1^{POS}$).[15] The results obtained are moderate (this compound inhibited SK-MEL-24 cell adhesion to FN-coated wells showing an IC_{50} of 1.2×10^{-4} M; it inhibited only 27% K562 cell adhesion to FN-coated wells at 10^{-5} M concentration), nevertheless suggested that the substrate bound to the receptor and could be used as a model to evaluate the influence of the scaffold stereochemistry on the interaction with integrins.

In order to gain further insight into the nature of ligand–receptor interactions, we focused our attention on the preparation of enantiomerically pure 3-bromo-4-methyl-5,6-dihydropyridin-2-ones to be exploited as rigid cores for novel RGD mimetics and studied the influence of the stereochemistry at the C6 of the scaffold on the bioactivity.

Ketenes have long been used in the synthesis of various heterocyclic compounds.[16] We have recently reported a good approach to the synthesis of 3-bromo-4-methyl-5,6-dihydropyridin-2-ones by treatment of 2-bromo-3-methyl-2-butenoyl chloride **2** and TEA in the presence of an appropriate Schiff base **3**.[12] Starting from the chiral imine **3**, deriving from the condensation of benzaldehyde and (*S*)-*p*-nitro-phenylethylamine,[17] dihydropyridin-2-ones (1′*S*,6*R*)-**4a** and (1′*S*,6*S*)-**4b** were obtained in 88% yield and 63:37 d.r. (Scheme 1). The diastereomeric mixture was easily separated by flash chromatography.

The presence of the bromide at C3 allows easy introduction of an appendage mimicking the aspartic acid function, while the nitro group on the aromatic ring is a useful precursor of the arginine mimic (Fig. 1). Compound **5a** was obtained by reaction of **4a** with allylamine at rt.[12] Treatment of **5a** with malonyl chloride in CH_2Cl_2 in the presence of TEA, afforded, in good yield, malonyl derivative **6a** (Scheme 2). Reduction of the nitro group



Scheme 1. Synthesis of 3-bromo-4-methyl-5,6-dihydropyridin-2-ones **4a**–**4b**.

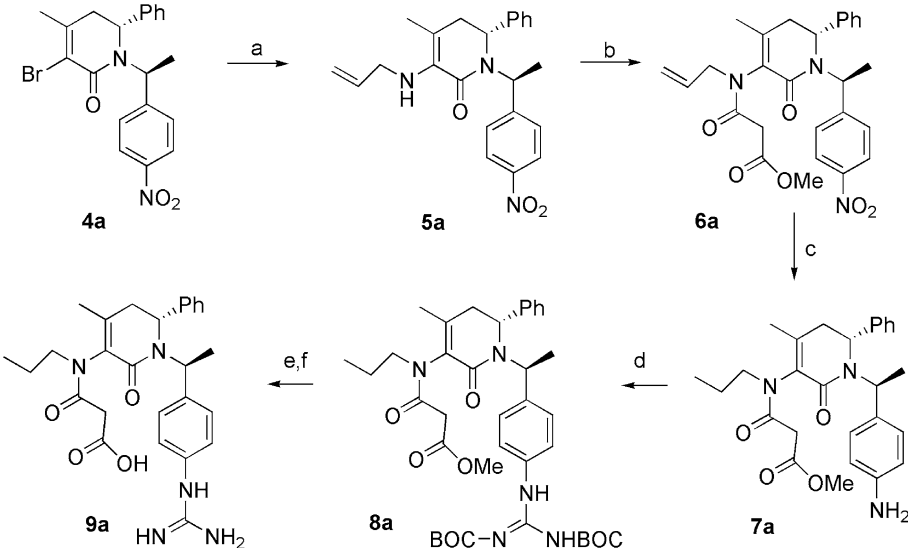
with H_2 on Pd/C allowed compound **7a** to be obtained. This compound, which has an amino function on the aromatic ring, was easily transformed into the Boc-protected guanidine derivative **8a** by treatment with carboxyamidine. Finally, hydrolysis of the methyl ester with LiOH, followed by treatment with trifluoroacetic acid to remove the Boc-protecting groups, afforded **9a**, which was further purified on a cationic exchange resin (Scheme 2).

The same reaction sequence was performed on compound **4b** to obtain the diastereoisomer **9b**.

To evaluate the activity of these optically active novel compounds in a cellular environment, we tested their ability to hinder initial cell attachment mediated by $\alpha_v\beta_3$ integrin, by using a cell adhesion assay. The integrin ligand fibronectin was immobilized on tissue culture plates and the adhesion of human melanoma cell line SK-MEL-24 ($\alpha_v\beta_3^{POS}$)[14] analyzed in the presence or absence of the peptidomimetics. The results are summarized in Table 1. The inhibitory activity of the well-known $\alpha_v\beta_3$ integrin antagonist H3528 was measured as a positive control.[18]

The data reported in the table show that no significant difference was found between the diastereomeric (6*R*)- and the (6*S*)-derivatives. Nevertheless compound **9a** with the (6*R*)-configuration seems to be slightly more active. This behaviour suggests that the configuration at C6 is not crucial for activity.

A likely interaction of compound **9a** with the $\alpha_v\beta_3$ integrin binding site is reported in Figure 2. The appendages of dihydropyridinone **9a** have been arranged by superimposition to the corresponding side chains of c(RGDf-N[Me]V) in the binding region of $\alpha_v\beta_3$ integrin extracellular domain.[19,20] The dihydropyridinone inserts into a crevice between the α and β subunits, disposing the aromatic ring linked to C6 towards the external space.



Scheme 2. Synthesis of compound **8a**. Reagents and conditions: (a) allylamine, 48 h, reflux; (b) TEA (1.5 equiv), malonyl chloride (1 equiv), CH_2Cl_2, 0 °C to rt; (c) Pd/C (30 mg/mmol), H_2 (1 atm), MeOH, rt; (d) *N*-Boc-1*H*-pyrazol-1-carboxyamidine (1.2 equiv), DMF, rt; (e) $LiOH{\cdot}H_2O$ (3 equiv), THF/MeOH/H_2O (3.6:1:1); (f) TFA, rt; (g) Dowex, MeOH, NH_4OH.

Table 1. SK-MEL-24 cell-fibronectin ($\alpha_v\beta_3$ mediated) adhesion inhibition results for compounds **9a** and **9b**

Compounds	Adhesion inhibition[a] IC_{50}
9a	$(8.1 \pm 1.1)\ 10^{-5}$ M
9b	$(1.1 \pm 0.3)\ 10^{-4}$ M
H3528	$(1.5 \pm 0.2)\ 10^{-10}$ M

[a] Concentration necessary to inhibit cell attachment to extracellular matrix coated with fibronectin to 50% of the control. Each value is the mean of at least three separate experiments carried out in quadruplicate.

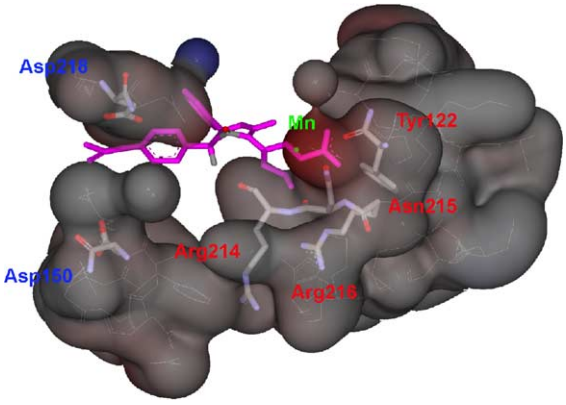


Figure 2. Possible interactions of 5,6-dihydropyridin-2-one **9a** with key residues in $\alpha_v\beta_3$ integrin receptor. Blue labels correspond to residues in the α_v subunit and red ones to residues in the β_3 chain.

3. Conclusion

In conclusion, although the activity values are not satisfactory, these compounds show a promising interaction with the receptor, confirming that 5,6-dihydropyridin-2-one could represent a useful scaffold in the preparation of a novel class of $\alpha_v\beta_3$ integrin. Attachment assays in a cellular environment, showed a small effect of the scaffold stereochemistry on the bioactivity. Further modifications will be addressed to the nature and length of the arginine and aspartate mimicking branches.

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