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Synthesis and biological evaluation of type VI β-turn templated RGD peptidomimetics

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Abstract—We report the design, synthesis, and binding affinities of a family of cyclic RGD peptides attached to type VI β-turn scaffolds. The analogues prepared exhibit interesting binding data to the isolated receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$. The results demonstrate the utility of these type VI β-turn scaffolds for the constraint of biologically relevant peptides.

A continuing area of research in many pharmaceutical companies and academic laboratories involves the rational transformation of a biologically important peptide ligand to a peptidomimetic lead compound followed by, in some cases, synthesis of a small molecule clinical candidate.[1] The initial stages of this type of research are usually carried out on the peptide by side-chain modifications, amino acid scans, insertion of non-natural amino acids, truncation of peptide sequences, and insertion of constraining scaffolds. The goal of these modifications is to optimize interactions between the modified ligand and the receptor of interest in an effort to understand what are required functional groups and what is the overall topology that leads to a selective, robust biological response. The method of going from peptide to drug candidate or tool compound has a proven track record in many research programs and the continued discovery of new building blocks for this type of research is of importance.

Recently, we reported synthesis and conformational analysis of a series of constrained eight-membered ring lactams (Fig. 1) based upon 7-amino-8-oxo-1,2,3,6,7-pentahydroazocine-2-carboxylic acid (**1**) as part of a program to identify novel scaffolds that adopt defined secondary structure. Conformational analysis of **2** (2*S*,7*S*) revealed an extended geometry of the pendent amide chains and **3** (2*R*,7*S*) is in a reverse turn conformation, more specifically, a type VIa β-turn in the solid state (X-ray crystal structure) and in water (NMR analysis). The type VIa β-turn is relatively rare, bearing the *cis*-amide bond typically found in proline-containing sequences and peptides that contain tertiary amides.[2,3]

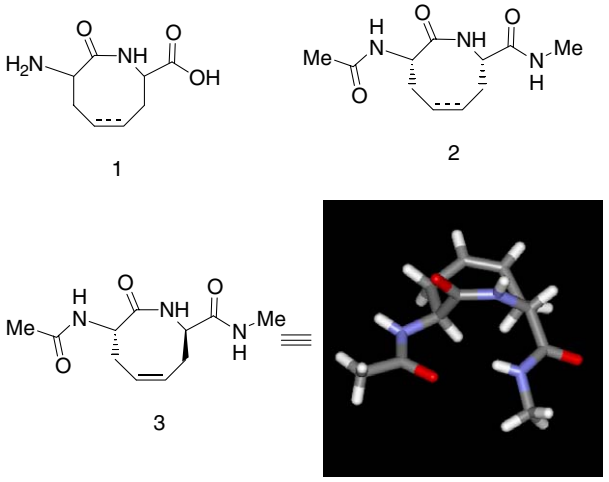

Figure 1. Various diastereomers of the 7-amino-8-oxo-1,2,3,6,7-pentahydroazocine-2-carboxylic acid scaffold (**1**), compound **3** is represented in Chemdraw and the stick representation based on an X-ray crystallography experiment.

Integrins are a family of hetero-dimeric glycoprotein cell surface receptors,[4] which regulate cell–cell and cell–matrix interactions.[5–7] These receptors mediate a variety of

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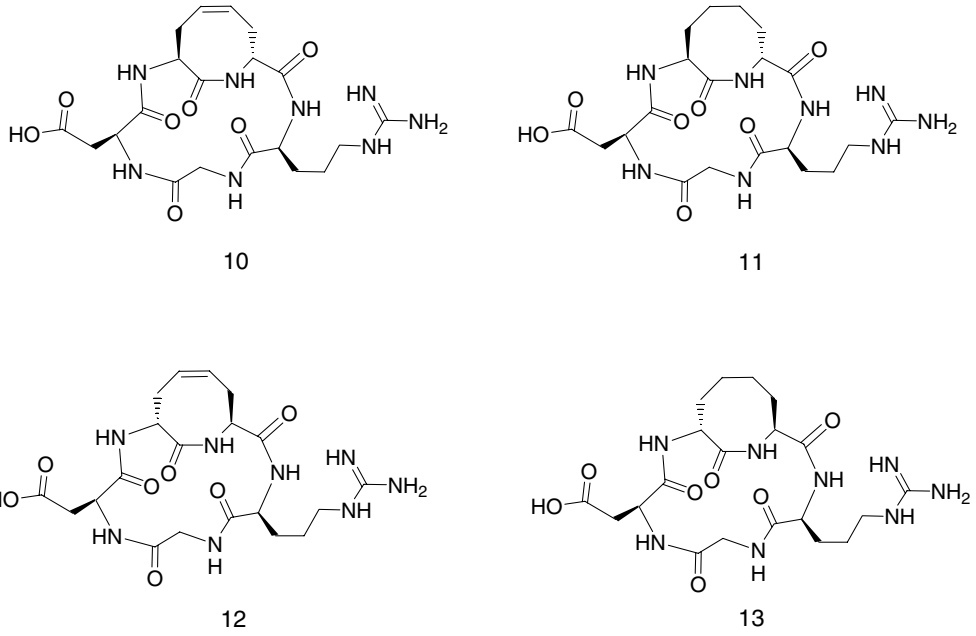


Figure 2. RGD peptides synthesized.

Table 1. All values are in micromolar

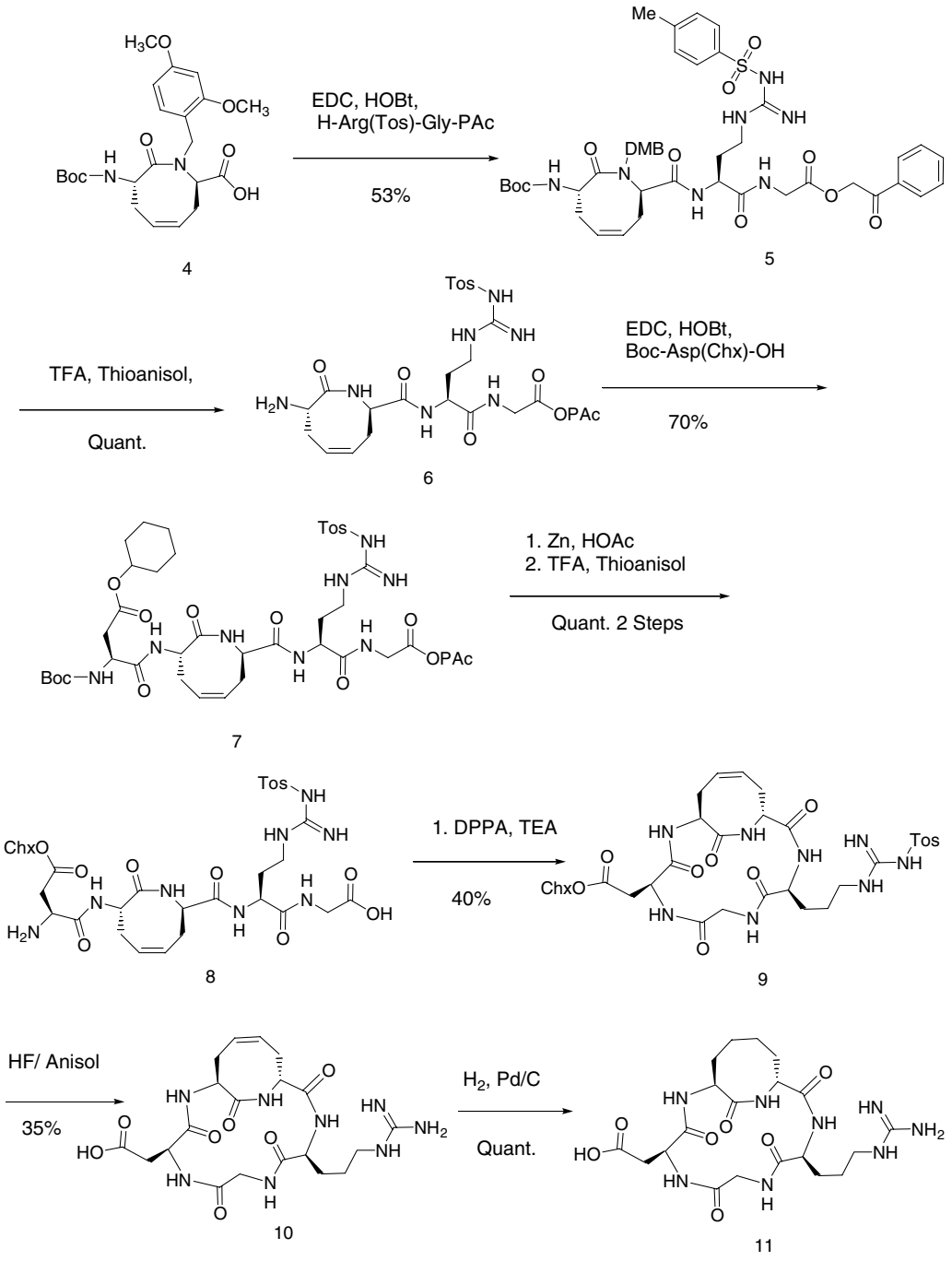
Compound	$\alpha_v\beta_3$	$\alpha_v\beta_5$
10	0.159	0.538
11	0.280	>5
12	0.925	>5
13	0.615	>5

cell adhesion events and signal transduction processes and are involved in tumor metastasis, angiogenesis, thrombosis, and osteoporosis.[8–11] The recognition site for many of the integrins that bind to the extra-cellular matrix is the tripeptide sequence, arginine-glycine-aspartic acid (RGD). The sequence was first identified in the protein fibronectin and has since been shown to be a recognition sequence in extra-cellular matrix proteins such as vitronectin and fibrinogen.[4] Subsequent research has shown that the RGD sequence can be embedded in small peptides or larger matrixes.

A wide variety of β-turn scaffolds based on amino acids and non-peptidic components have been incorporated into RGD-containing peptides in an effort to elucidate the essential biological conformations of integrin receptor antagonists.[12] Much progress has been made in this area of research culminating with the synthesis of possibly the most interesting RGD-containing peptide/peptidomimetic being Cilengitide (EMD-121974) (c[RGDf*N*MeV]) which was recently co-crystallized with the extra-cellular segment of the integrin $\alpha_v\beta_3$. With that in mind we inserted 7-amino-8-oxo-1,2,3,6,7-pentahydroazocine-2-carboxylic acid scaffolds into an RGD peptide in an effort to evaluate the effect of the type VI β-turn on the overall activity and for selectivity of the peptides to $\alpha_v\beta_5$ and $\alpha_v\beta_3$ interactions.

Synthesis of the peptides (see Scheme 1) was accomplished by allowing compound **4**[2,3] (*S*,*R*) to react with the protected dipeptide H-Arg(Tos)-Gly-OPAc using EDC/HOBt coupling conditions yielding tetrapeptide **5**. This compound was treated with TFA and thioanisol to remove the 2,4-dimethoxy benzyl and Boc protecting groups providing **6**. Tetrapeptide **6** was coupled to Boc-Asp(Chx)-OH again employing EDC/HOBt followed by deprotection of the phenylacyl ester and the Boc protecting groups to yield **8**. Cyclization of pentapeptide **8** was accomplished using DPPA in DMF to afford cyclic peptide **9** which was treated with hydrofluoric acid to provide **10**. Reduction of the olefin using hydrogen and Pd/C yielded **11**. The same reaction sequence was used starting with the (*R*,*S*) diastereomer of **4** to yield compounds **12** and **13**.

Compound **10** (see Table 1) inhibited the vitronectin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ interactions to the extent of 160 and 530 nM, respectively.[14] Reduction of the olefin (**11**), which in model systems allows for relaxation of the ring and switching of the peptide backbone from a type VIa β-turn to a type VIb β-turn, causes a modest loss



Scheme 1.

in inhibitory activity at $\alpha_v\beta_3$ but completely abolishes the inhibition of the vitronectin-$\alpha_v\beta_5$ interaction. Compounds **12** and **13** exhibit modest inhibition of the vitronectin-$\alpha_v\beta_3$ interaction and relatively no $\alpha_v\beta_5$ activity. However when the (*R*,*S*) scaffold is incorporated in compounds **12** and **13**, the saturated scaffold causes an increase in the inhibition of the vitronectin-$\alpha_v\beta_3$ interaction, opposite of the results with the (*S*,*R*) scaffold in compounds **10** and **11**. These results demonstrate the utility of this 7-amino-8-oxo-1,2,3,6,7-pentahydroazocine-2-carboxylic acid system in peptidomimetic research. Changing the stereochemistry and saturation of the scaffold has varying effects on the overall topology of the peptide, producing subtle but unique biological responses for each change in the scaffold.

The final calculated structures for compounds **10**–**13** compared to Cilengitide are shown in Figure 3. While all these compounds were able to mimic the ASP218/ARG and acid/MIDAS site orientations found in the RGD structure, the backbone structure that lies in the shallow pocket between these two sites differs considerably from RGD. Overall, the RGD ligand is able to lay flatter and thus in closer contact with the protein surface.[15]

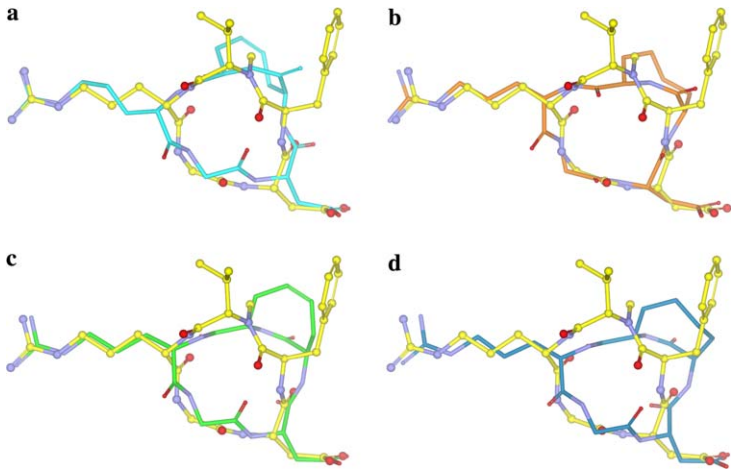


Figure 3. Compounds **10(a)**, **11(b)**, **12(c)**, and **13(d)** overlaid with Cilengitide (yellow).

Acknowledgments

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