# Novel RGD Peptidomimetics Embedding 1,2,3-Triazole as Central Scaffold; Synthesis and $\alpha_v\beta_3$ Integrin Affinity

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**Abstract:** Ten new RGD (Arginine-Glycine-Aspartic acid) peptidomimetics have been synthesized and screened for their affinity to $\alpha_v\beta_3$ integrin receptor. Arginine and Aspartic acid mimetic subunits were connected through 1,2,3-triazole as central scaffold by click chemistry, affording the final products with good yields. Among them, compounds **3f-j** exhibited high affinity to the receptor,with $IC_{50}$ in the low nanomolar range, comparable to that of the reference compound Cilengitide.

**Keywords:** RGD peptidomimetics, $\alpha_v\beta_3$ integrin, Osteoporosis, 1,2,3-Triazole, Click chemistry.

## INTRODUCTION

RGD peptides are a well known class of integrin membrane receptor ligands [1]. Among the integrin superfamily, $\alpha_v\beta_3$ is overexpressed on a variety of cells during proliferation, and plays a role in several relevant processes based on cell-cell and cell-matrix adhesion. This integrin recognizes molecules containing the peptide sequence RGD; therefore, it is a priviledged target for antagonists containing this sequence, which are expected to have utility in the treatment of several human diseases, like tumor induced angiogenesis, restenosis and osteoporosis.

During the last two decades, the understanding of the structural requirements for optimizing the receptor affinity of RGD analogs has given impetus to the development of a great number of potent antagonists. Specificity and efficacy of the molecular recognition process depend upon the conformational presentation of the peptide backbone, which determines the optimal orientation of the two arginine and aspartic acid pharmacophores for selective binding to the receptor [2,3].

Later on, small non-peptide molecules mimicking the RGD structure have been proposed by several researchers, with the purpose to obtain drug-like compounds with improved stability and bioavailability comparing to the parent peptides [4]. Several different heterocyclic scaffolds have been proposed as a core structure substituting glycine between the basic and acidic moieties, with the purpose to guarantee their optimal distance and spatial orientation for affinity to the receptor.

We have undertaken the synthesis of a series of RGD peptidomimetics containing 1,2,3-triazole as central scaffold, considering this heterocycle particularly attractive, since a diverse array of structural types can be rapidly prepared and evaluated through the facility and generality of the "click chemistry" [5].

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We focused our interest on a class of structures containing 2-aminopyridine at the *N*-terminus and *N*-α-arylsulfonamide-*N*-β-diaminopropionic acid at the *C*-terminus, which have been developed by others as potential therapeutics for osteoporosis, because $\alpha_v\beta_3$ is highly expressed in the osteoclasts but not present in the osteoblasts [6-10]. Moreover, RGD mimetics containing the amidino-like aminopyridine in place of arginine have been claimed to show high receptor affinity and selectivity as well as bioavailability in rats [11,12]. In this article, we report on the synthesis and $\alpha_v\beta_3$ affinity of ten RGD mimetics, where the two pharmacophores are connected through linkers containing triazole in different positions. During the preparation of our paper, another group of researchers reported on a number of RGD analogs, where arginine and aspartic acid mimetics have been connected by click chemistry [13].

## RESULTS AND DISCUSSION

### Synthesis

The building blocks consisting of arginine mimetic subunits **1a-f** were prepared as described in the Scheme **1**, following already described methods: **1a** was prepared from 2-amino-5-nitropyridine by reduction, followed by the aromatic azide formation [14]. **1b-d** were obtained by Sonogashira reaction [15] from 2-amino-4-or 5-bromopyridine or 7-bromo-azaindole. **1e** and **1f** were obtained by arylation of propargylamine with 2-amino-6-bromopyridine (Heck reaction), followed by catalytic transfer hydrogenation and azide formation [16]. The aspartic acid mimetic building blocks **2a-e** were obtained from 2-L-arylsulfonamido-diaminopropionic acid t-butyl ester **4** or from the glutamic acid derivative **5** by coupling with the desired residues, as described in the Scheme **2**.

The assembly of the subunits containing either azido or alkyne groups to afford the final products **3a-j** was performed by the Huisgen 1,3-dipolar cycloaddition [5], as described in the Scheme **3**. The clean and chemoselective click chemistry allowed us to easily obtain several analogs with 95% purity and good yields. Physico-chemical data are shown in Table **1**.

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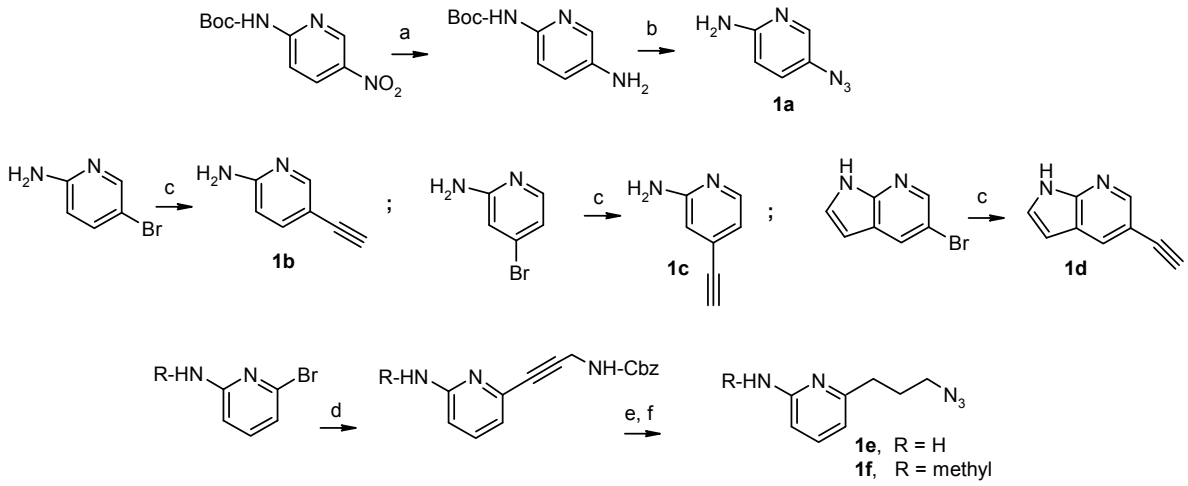
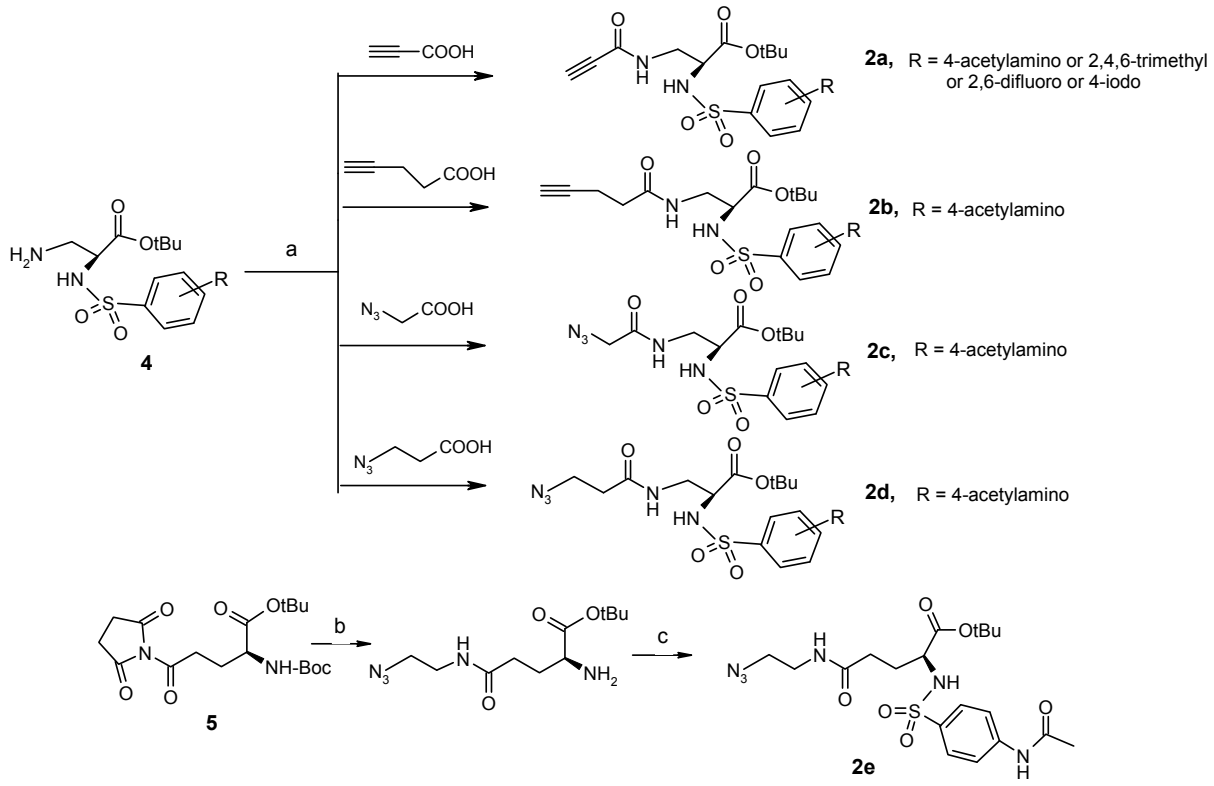
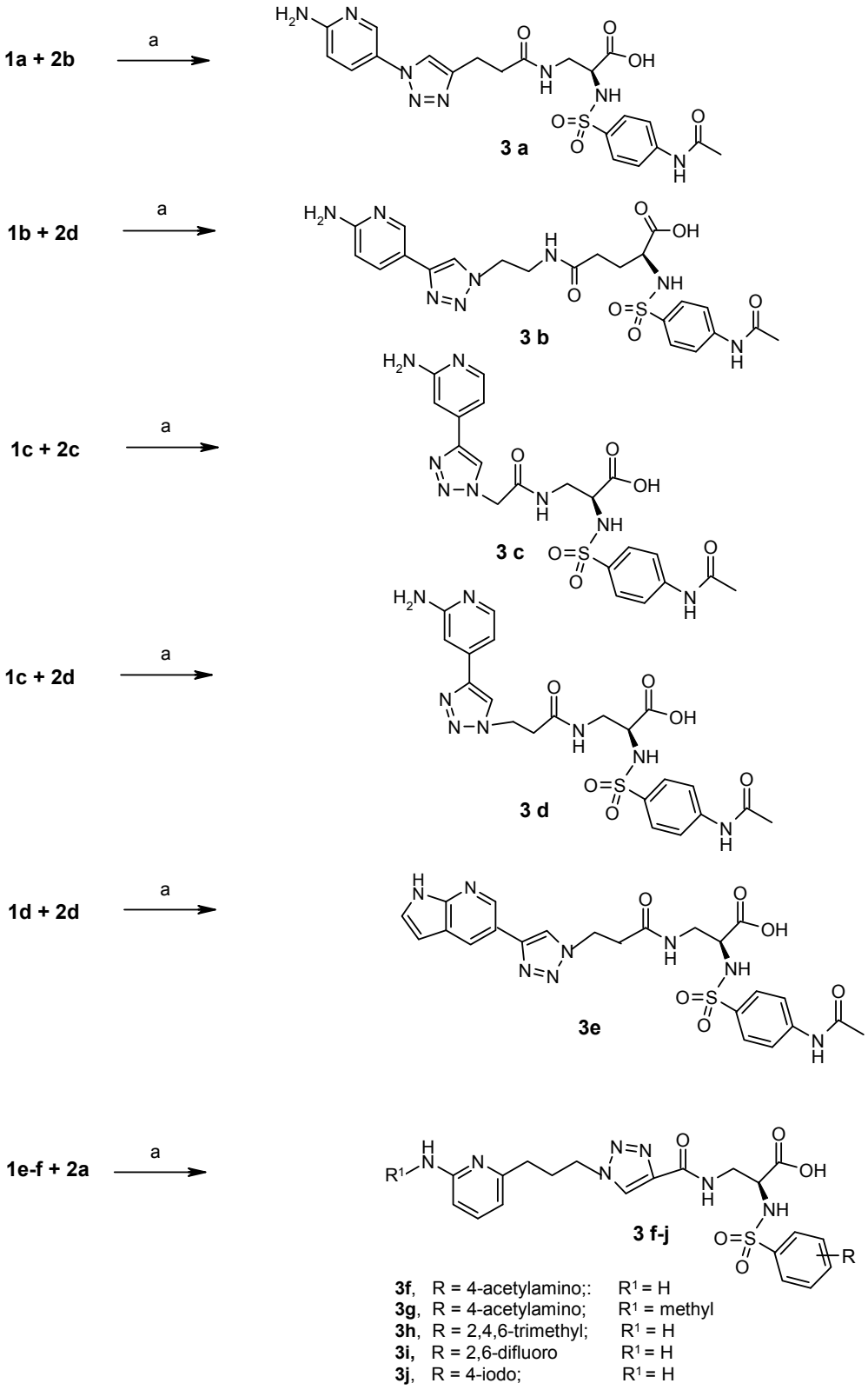



**Scheme 1.** Synthesis of arginine mimetic subunits **1a-f**. Reagents and conditions: a, ammonium formate, 10% Pd/C, 1h, 86%; b, i, $TMSiN_3$, $tBuNO_2$, 48 h, ii, TFA/DCM; c, i, Ethynyltrimethylsilane, $(Ph_3P)_2PdCl_2$, CuI, 2.5 h; ii, MeOH, $K_2CO_3$, 3 h, yields around 50% for two steps; d, *N*-Cbz-propargylamine, $(Ph_3P)_2PdCl_2$, CuI, TEA, 100°C, 3 h, 80.3%; e, same as a, 2 h; f, $TfN_3$, overnight, 78.3% for two steps.




**Scheme 2.** Synthesis of aspartic acid mimetic subunits **2a-e**. Reagents and conditions: a, HOAT, DCC, DIPEA, overnight; b, i, 2-azidoethylamine, DIPEA, 2 h, ii, 4 M HCl in dioxane, 77.4% for two steps; c, 4-acetamidobenzenesulfonyl chloride, DIPEA, overnight, 88.7%.




**Scheme 3.** Synthesis of RGD peptidomimetics **3 a-j**. Reagents and conditions: a, i, solvent water/t-butylalcohol, 1M sodium ascorbate (0.1 equiv), 0.5 copper sulfate (0.01 equiv), ii, 50% TFA/DCM, total yield > 80%.

**Table 1. Physico-Chemical Characteristics of Compounds 3a-3j**

| Entry | HPLC Rt, min([a]) | Esi mass [M+H$^+$] | $^1$H-NMR |
|---|---|---|---|
| **3a** | 9.6 (15) | 517.00 | ($D_2O$): δ: 8.29 (d, J=2.3Hz, 1H), 8.24, 8.21 (dd, J=2.5and 9.7Hz, 1H), 8.13(s, 1H), 7.73, 7.61 (dd, J=8.8 and 37.4 Hz, 4H), 7.18 (d, J=9.7Hz, 1H), 3.95 – 3.90 (m, 1H), 3.59-3.55 (dd, J=4.5 and 14.1Hz, 1H), 3.30-3.27 (dd, J=8.8 and 14.1Hz, 1H), 2.95 (t, J=7.2Hz, 2H), 2.59- 2.44 (m, 2H), 2.11 (s, 3H). |
| **3b** | 11.6 (15) | 531.17 | ($D_2O$): δ: 8.29 (s, 1H), 8.21- 8.18 (dd, J=2.2 and 9.4 Hz, 1H, ), 8.15 (s, 1H), 7.72 -7.61 (dd, J=8.8 and 33.1 Hz, 4H), 7.10 (d, J=9.2 Hz, 1H), 4.59 (t, J=5.6 Hz, 2H), 3.71 – 3.65 (m, 3H), 2.27 (t, J= 7.3 Hz, 2H), 2.18 (s, 3H), 2.02-1.90 (m, 1H), 1.79-1.68 (m, 1H). |
| **3c** | 11.4 (15) | 503.15 | (DMSO-$d_6$+ $D_2O$): δ:8.64 (s, 1H), 7.91(d, J=6.0Hz, 1H), 7.67 (s, 4H), 7.37 (s, 1H), 7.19 (d, J=6.0Hz, 1H), 5.09 (d, J=6.0Hz, 2H), 3.86 (t, J=6.6 Hz, 1H), 3.43-3.38 (m, 1H), 3.23-3.14 (m, 1H), 2.01 (s, 3H). |
| **3d** | 11.9 (15) | 517.16 | (DMSO-$d_6$ + $D_2O$): δ : 8.60 (s, 1H), 7.90 (s, 1H), 7.67 (d, J=4 Hz, 4H), 7.25 (s, 1H), 7.12 (d, J=5.4 Hz), 4.50 (t, J=6.9 Hz, 2H), 3.83 (t, J=6.8 Hz, 1H), 3.34-3.28 (m, 1H ), 3.15-3.08 (m, 1H), 2.73-2.60 (m, 2H), 2.04 (s, 3H). |
| **3e** | 7.8 (20) | 541.16 | (DMSO-$d_6$ + $D_2O$): δ: 8.68 (d, J=1.9 Hz, 1H), 8.40 (s, 1H), 8.34 (d, J=1.6 Hz, 1H), 7.70 (d, J=4.0 Hz, 4H), 7.47 (d, J= 3.5 Hz, 1H), 6.49 (d, J=3.4 Hz, 1H), 4.53 (t, J=7.0 Hz, 2H), 3.87 (t, J=6.5 Hz, 1H), 3.37-3.30 (m, 1H), 3.20-3.13 (m, 1H), 2.71-2.64 (m, 2H), 2.05 (s, 3H). |
| **3f** | 8.3 (18) | 531.16 | ($D_2O$): δ: 8.19 (s, 1H), 7.74-7.68 (m, 3H), 7.41 (d, J=8.6Hz, 2H), 6.75 (d, J=9.0Hz, 1H), 6.63 (d, J=7.2Hz, 1H), 4.55 (t, J=6.0Hz, 2H), 4.20-4.16 (m, 1H), 3.79 -3.74 (dd, J=14.2 and 4.0Hz, 1H), 3.51-3.46(dd, J=14.2 and 10.0Hz, 1H), 2.80 (t, J=8.5Hz, 2H), 2.39 (q, J=8.5Hz, 2H), 2.10 (s, 3H). |
| **3g** | 8.2 (19) | 545.19 | ($D_2O$): δ: 8.17 (s, 1H), 7.69 (t, J=8.9Hz, 1H), 7.71 (d, J=8.8Hz, 2H), 7.41(d, J=8.8Hz, 2H), 6.72 (d, J=8.9Hz, 1H), 6.59 (d, J=7.1Hz, 1H), 4.55 (t, J=6.0Hz, 2H), 4.20-4.16 (m, 1H), 3.79-3.74 (dd, J=14.2Hz and 4Hz, 1H), 3.51-3.46 (dd, J=14.2 and 10.0Hz, 1H), 2.93 (s, 3H), 2.83 (t, J=7.2Hz, 2H), 2.40 (q, J=6.6Hz, 2H), 2.15 (s, 1H). |
| **3h** | 7.5 (30) | 516.09 | (DMSO-$d_6$): δ: 8.51 (s,1H), 8.26 (t, J=6.0 Hz, 1H), 7.86 (d, 1H), 7.74 (t, J=8.6 Hz, 1H), 6.87 (s,2H), 6.73-6.65 (dd, J=8.6Hz, 2H), 4.47 (t, J=6.9 Hz, 2H), 3.98-3.91 (m, 1H), 3.60-3.51 (m, 1H), 3.45-3.34 (m, 1H), 2.68 (t, J=7.2 Hz, 2H), 2.53 (s, 6H), 2.21 (q, J=7.2Hz, 2H), 2.19 (s, 3H). |
| **3i** | 8.5 (22) | 510.14 | ($D_2O$): δ: 8.26 (s,1H), 7.74-7.71 (dd, J=7.3 Hz, 1H), 7.50-7.41 (m, 1H), 6.98 (t, J=9.1Hz, 2H), 6.77 (d, J=7.5Hz, 1H), 6.65 (d, J=7.5Hz, 1H), 4.60 (t, J=6.5Hz, 2H), 4.40-4.35 (m, 1H), 3.85-3.79 (dd, J=14.1 and 4.2Hz, 1H), 3.64-3.56 (dd, J=14.1 and 9.5Hz, 1H), 2.84 (t, J=7.3Hz, 2H), 2.42 (q, J=7.2 Hz, 2H). |
| **3j** | 8.6 (28) | 600.04 | (DMSO-$d_6$), δ: 8.52 (s, 1H), 8.27 (t, J=6.0Hz, 1H), 8.23 (d, J=8.2Hz, 1H), 7.80 (s, 2H), 7.76 (t, J=8.5Hz, 1H), 7.76 -7.50 (dd, J=8.5 and 84.6Hz, 4H), 6.83 (d, J=8.8Hz, 1H), 6.71 (d, J=7.3Hz, 1H), 4.49 (t, J=6.9Hz, 2H), 4.00 (t, J=6.2Hz, 1H), 3.62-3.50 (m, 1H), 3.40-3.25 (m, 1H), 2.74 (t, J=7.3Hz, 2H), 2.30 (q, J=7.6Hz, 2H). |

[a]Acetonitrile percentage in the mobile phase is indicated in brackets.

### Affinity to $\alpha_v\beta_3$ Integrin

The series of analogs synthesized can be divided in two groups. The first group comprises compounds **3a-e**, which have in common the *C*-terminus, but differ for the *N*-terminus, where the triazole scaffold is directly linked to aminopyridine in different positions. None of these compounds proved to have significant affinity to the receptor. We observed that, in the structures of this group, the two heterocycles are very close together; thus, it is plausible that the steric bulk and/or rigidity of the amidino-like moiety has restricted the available space in the site of the receptor too severely, resulting in a mismatched conformation.

As a consequence of these considerations, we designed a second group of compounds, **3f-j**, where the triazole is linked to aminopyridine through a flexible methylene chain, and they differ from each other for the aryl-substituted *C*-terminus. In this case, we hypothesized the simple carbon chain (similar to that of arginine in the parent peptide) to create some degrees of freedom, allowing the basic pharmacophore to assume the proper spatial orientation into the receptor site. As shown in Table **2**, these analogs, with the exception of the highly insoluble **3j**, exhibited excellent affinity toward $\alpha_v\beta_3$ integrin, comparable to Cilengitide, c(RGDfMeV). Since this integrin has been shown to mediate adhesion of osteoclasts to the bone matrix, the structures of this group can be considered interesting leads for further development of drug-like compounds, potentially useful against osteoporosis.

## MATERIALS AND METHODS

Amino-bromo- or amino-nitro-pyridines, bromo-azaindole, substituted benzenesulfonyl- chlorides, propiolic and pentynoic acids, protected glutamic acid were purchased from Sigma-Aldrich. *N*-α-Boc-*N*-β-Allo*c*-L-diaminopropionic acid was purchased from PolyPeptide Laboratories, and, after protection as t-butyl ester and removal of Boc, was coupled with the arylsulfonyl chloride to obtain compound **4**. 2-methylamino-6-bromopyridine was prepared from the 2-

**Table 2. Inhibition of Echistatin Binding to $\alpha_v\beta_3$ Integrin Receptor**

| Entry | $IC_{50}$, nM |
|---|---|
| **Cilengitide** | 18.9 ± 3.1 |
| **3a** | ›1000 |
| **3b** | ›1000 |
| **3c** | ›1000 |
| **3d** | ›1000 |
| **3e** | ›1000 |
| **3f** | 14.28 ± 2.3 |
| **3g** | 15.70 ± 0.7 |
| **3h** | 13.60 ± 0.9 |
| **3i** | 17.20 ± 2.1 |
| **3j** | 52.80 ± 0.7 |

amino-6-bromopyridine with paraformaldeyde by reductive alkylation. Microwave-assisted reactions were performed in a CEM MW instrument. When necessary, crude products were purified by flash chromatography on silicagel Merck 230-400 mesh. Purity of compounds was detected by RP-HPLC (column: Gemini C18, 5 µ, 4.6 X 250, Phenomenex®; mobile phase, acetonitrile/water + 0.1% TFA). Mass spectra were recorded on a Bruker Micro-Tof instrument. 1H-NMR spectra were recorded on a AC300 Bruker instrument.

## Synthesis of Peptidomimetics

### *General Procedure*

The desired couple of building blocks **1a-f** and **2a-e**, containing an azido or an alkyne functional group, respectively, were dissolved with a mixture of t-BuOH / water , 0.1 equiv of sodium ascorbate was added followed by 0.01 equiv of copper sulfate. In the case of **3a-e**, a three fold excess of salts and repeated microwave irradiation (100 W for two minutes) were necessary to complete the reaction. After about 2h, solvent was evaporated and the crude products **3a-j** purified by flash chromatography. Removal of the tert-butyl ester with 50% TFA in DCM afforded the final products with up to 80% yields for the two steps, and purity >95% on analytical RP-HPLC.

## Binding to $\alpha_v\beta_3$ Integrin

Binding tests were performed according to the method of Kumar *et al.* [17], following the procedure already published [18].

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