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# Pyridone derivatives as potent and selective VLA-4 integrin antagonists

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**Abstract**—A novel series of pyridone inhibitors has been identified through pharmacophore analysis, as potent antagonists of VLA-4.


VLA-4 (Very Late Antigen-4, α4β1, or CD49d/CD29) is a member of the superfamily of transmembrane glycoprotein integrins which is made up of α- and β-heterodimers and is expressed on all leucocytes except platelets.[1–3] VLA-4 binds to an alternatively spliced segment of fibronectin (CS-1) on extracellular matrix and to vascular cell adhesion molecule-1 (VCAM-1) on endothelium. VCAM-1 is expressed on vascular endothelial cells in response to proinflammatory cytokines and the VCAM-1/VLA-4 binding interaction has been shown to be critical for lymphocyte migration to extravascular tissues.[4] Antibodies against VLA-4 have been shown to block leucocytes infiltration and prevent tissue damage in inflammatory disease models of asthma,[5] rheumatoid arthritis,[6] multiple sclerosis (MS)[7] and inflammatory bowel disease.[8] Orally active small molecule inhibitors of VLA-4 might therefore serve as useful agents in the treatment of these disease states. Indeed, a small molecule antagonist of VLA-4 has recently demonstrated efficacy in an acute model of MS.[9]

Recently, we identified a series of C-ring heterocyclic ureas as antagonists of VLA-4 (Table 1). While the urea moiety, and the position and length of the carboxylate linker were essential for potency (data not shown) the SAR around the C-ring attracted most interest. Although direct comparisons could not be made, initial SAR around the C-ring heterocycle appeared to suggest that a nitrogen at the 2-position was beneficial for inhibitory potency (e.g., **1** and **2**). Replacement of the nitrogen atom for oxygen also appeared to be detrimental for potency (cf. **5** and **6**).

Given the structural similarity of **1** and **5** with the known[11] and potent VLA-4 antagonists **7** and **8**, together with the finding that both series demonstrate similar B-ring SAR (cf. **1** and **5** with **7** and **8**), we decided to overlay the two series in an attempt to define a common pharmacophore (Fig. 1).

The molecular overlay of **5** and **8** may explain the similarity in SAR between the two series, with excellent alignment of both the urea and carboxylate moieties. Furthermore, the nitrogen at the 2-position of the C-ring of **5** is orientated in the same direction, but not identical position, to the lone pair of the carbonyl moiety of **8**. Although the published pharmacophore of **8** postulates the amide moiety acts as a hydrogen bond donor,[12] given the excellent overlay with **5**, we decided to explore the possibility that the amide might in fact behave as a H-bond acceptor (Fig. 2).

*Keywords*: VLA-4; Molecular overlays; Pharmacophore; Pyridone; Ureas; GASP.
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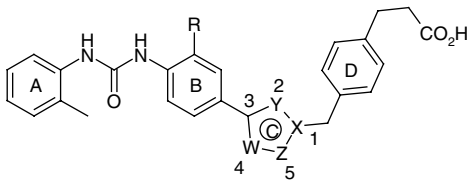
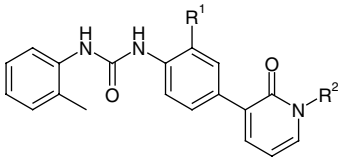
**Table 1.** Heterocyclic C-ring SAR



| Compound | W | X | Y | Z | R | p$K_i$ α4β1[10] |
|---|---|---|---|---|---|---|
| **1** | C | N | N | C | H | 6.6 |
| **2** | N | N | N | C | H | 6.3 |
| **3** | N | N | C | C | H | <4 |
| **4** | C | N | C | N | H | <6 |
| **5** | C | N | N | C | MeO | 7.6 |
| **6** | N | C | O | N | MeO | <6 |

**Table 2.** SAR for a series of pyridone analogues



| Compound | $R^1$ | $R^2$ | p$K_i$ α4β1 |
|---|---|---|---|
| **9** | H | Ph–4-$(CH_2)_2CO_2H$ | <6 |
| **10** | H | $CH_2$–Ph–4-$(CH_2)_2CO_2H$ | 8.7 |
| **11** | OMe | $CH_2$–Ph–4-$(CH_2)_2CO_2H$ | 9.3 |
| **12** | H | $CH_2$–Ph–3-$(CH_2)_2CO_2H$ | 8.4 |
| **13** | H | $CH_2$–Ph–2-$(CH_2)_2CO_2H$ | 6.4 |
| **14** | H | $CH_2$–Ph–4-$CH_2CO_2H$ | 6.6 |
| **15** | H | $CH_2$–Ph–4-$CO_2H$ | 6.5 |

A small subset of compounds, with variations in linker length and position, were designed and analysed for molecular overlap with compound **8**. Several of these compounds were selected for synthesis based on alignment of the proposed pharmacophoric features (Fig. 3).

Disappointingly, despite the excellent overlay the directly linked pyridone analogue **9** was considerably less active, possibly due to the rigid nature of this molecule and its inability to achieve the required bioactive conformation (Table 2). Gratifyingly, however, analogue **10**

**Table 3.** Cross screening data for pyridone **10**

| Integrin | p$K_i$ |
|---|---|
| α4β1 | 8.7 |
| α4β7 | <5 |
| α5β1 | <5 |
| αVβ3 | <5 |
| αVβ6 | <5 |
| αIIβ3 | <5 |

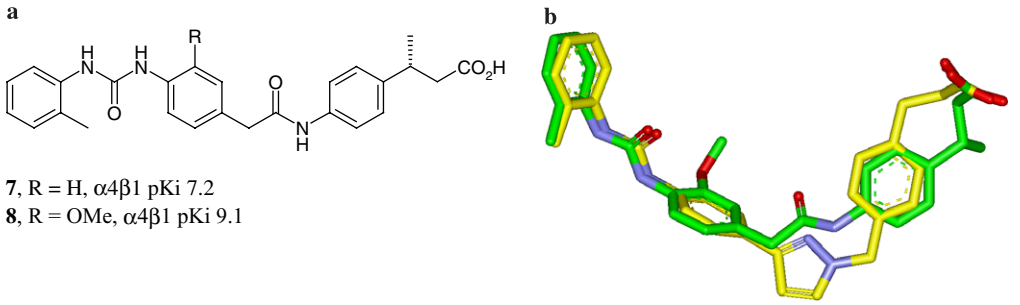



**Figure 1.** (a) Amide antagonists **7** and **8**. (b) GASP molecular overlay of **8** (yellow) and **5** (green).

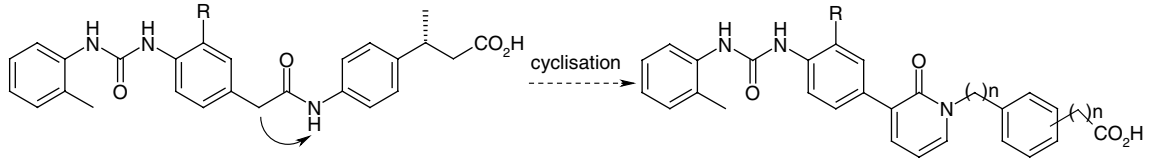



**Figure 2.** Cyclisation of amides to interrogate a hydrogen bond acceptor hypothesis.

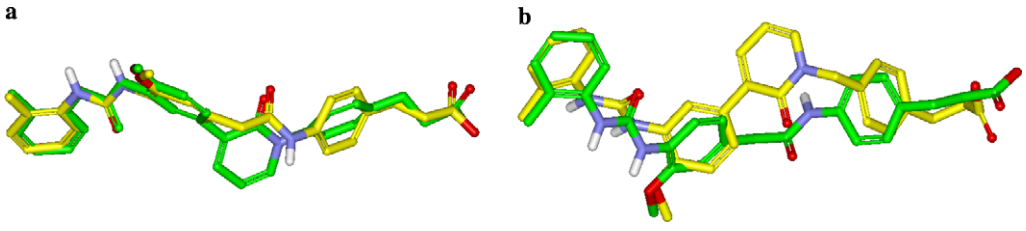
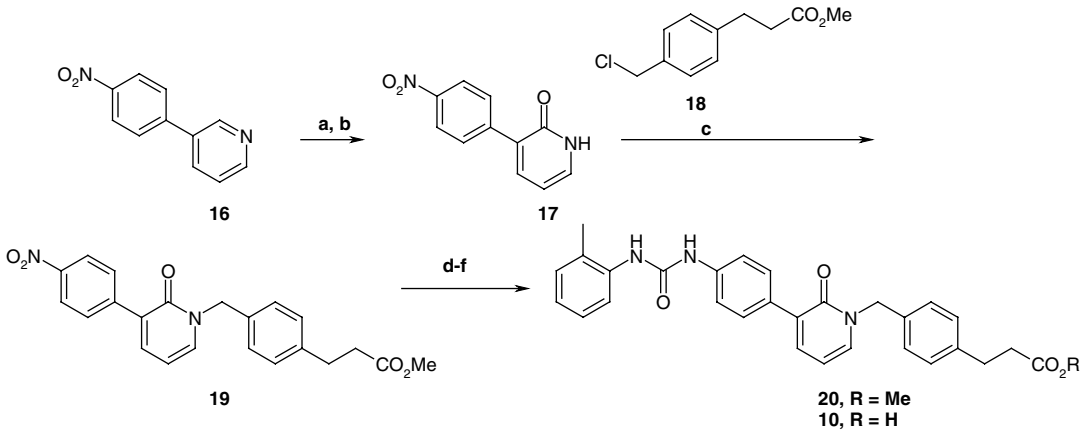



**Figure 3.** (a) Molecular overlay of 8 (yellow) and 9 (green). (b) Molecular overlay of **8** (yellow) and **10** (green).




**Scheme 1.** Reagents: (a) *m*CPBA, THF, 25 °C (58%); (b) i—$Ac_2O$, reflux; ii—HCl, reflux, (80%); (c) $Cs_2CO_3$, DMF, 25 °C (90%); (d) 10% Pd/C, EtOH, $H_2$ (1 atm), 25 °C (50%); (e) *o*-tolyl isocyanate, DCM, 25 °C (80%); (f) 0.5 N aq LiOH, THF, 25 °C (100%).

displayed excellent inhibitory potency and introduction of a methoxy group into the B-ring gave further enhancement in potency (cf. **10** and **11**). The increased potency observed for the pyridone **10** relative to the amide **7** is also noteworthy, suggesting that the carbonyl of the pyridone may be held in its bioactive conformation. Although the meta isomer **12** displays comparable potency to the para isomer **10**, in general the position and length of the linker is critical for optimal potency (cf. **10** and **13**, **14** and **15**).

Cross screening of **10** against a range of integrin receptors indicated excellent selectivity (Table 3). Additionally, cross screening of **10** against a diverse panel of 50 receptors and ion channels[13] indicated $\leqslant$15% inhibition at 1 μM (data not shown).

The pyridone analogues were prepared via a short concise synthesis[14] starting with the oxidation of the known pyridine **16**[15] with *m*-chloroperoxybenzoic acid which afforded the corresponding pyridine *N*-oxide. Treatment of the *N*-oxide with acetic anhydride at reflux afforded, after hydrolysis, the corresponding pyridone **17**. Selective N-alkylation of **17** with the chloride **18**[16] in the presence of caesium carbonate afforded the ester **19**. Hydrogenation of the nitro group afforded the corresponding aniline, which was treated with *o*-tolyl isocyanate to afford the urea **20**. Hydrolysis with lithium hydroxide provided the desired analogue **10** in good overall yield (Scheme 1).

In summary, molecular overlay studies of our initial leads with known VLA-4 antagonists have led to hypothesis of a novel pharmacophore. Subsequent design and synthesis of analogues exploiting this hypothesis has afforded a novel series of potent and selective VLA-4 antagonists.

## References and notes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10. A Jurkat J6 Scintillation Proximity Assay was used to investigate the interaction of the integrin VLA-4 (Very Late Antigen-4; α4β1; CD49d, CD29) expressed on the Jurkat J6 cell membrane with test compounds. J6 cells (1 million cells/well) were allowed to coat wheat germ agglutinin-coated SPA beads (Amersham, 1 mg/well) in assay buffer containing 50 mM HEPES, 100 mM NaCl and 1 mM $MnCl_2$ (pH with 4 M NaOH to 7.5). Tritiated $^3$H Standard Compound A (1–3 nM final assay concentration) and test compounds were dissolved in an appropriate solvent and diluted in assay buffer. Data are presented as means of p$K_i$. Standard compound A is (2*S*)-3-[4-({[4-(aminocarbonyl)-1-piperidinyl]carbonyl}oxy)-phenyl]-2-[((2*S*)-4-methyl-2-{[2-(2-methylphenoxy)acetyl]amino}pentanoyl)amino] propanoic acid potassium salt which is described in patent application WO 00/37444. Compounds were assayed in duplicate, a four-parameter curve fit being applied. The equilibrium dissociation constant for each compound was calculated according to the method of Cheng and Prusoff (*Biochem. Pharmacol.* **1973**, *22*, 3099).
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