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Bioorganic & Medicinal Chemistry Letters 16 (2006) 5538–5541
Bioorganic & Medicinal Chemistry Letters

Pyridone derivatives as potent, orally bioavailable VLA-4 integrin antagonists

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Received 14 June 2006; revised 31 July 2006; accepted 8 August 2006
Available online 22 August 2006

Abstract—A series of pyridone-*N*-benzyl-propanoic acids have been optimised to afford potent orally bioavailable VLA-4 antagonists.

VLA-4 (Very Late Antigen-4, α4β1, or CD49d/CD29) is a member of the superfamily of transmembrane glycoprotein integrins made up of α- and β-heterodimers expressed on all leukocytes except neutrophils and 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 leukocyte infiltration and prevent tissue damage in inflammatory disease models of asthma,[5] rheumatoid arthritis,[6] multiple sclerosis (MS),[7] and inflammatory bowel disease.[8] Recently, humanised monoclonal anti-α4 antibody (Tysabri) has demonstrated efficacy for MS and Crohn's disease in phase III clinical trials.[9] Orally active small molecule inhibitors of VLA-4 might therefore serve as useful agents in the treatment of these diseases (Scheme 1).

Recently, we disclosed a novel series of pyridone containing VLA-4 antagonists.[10] Herein we report the subsequent SAR optimisation and in vivo properties of these compounds. Previously it had been reported that the presence of an ortho methyl group in the terminal aromatic ring of the urea gave optimal inhibitory potency.[11] Indeed, we had found incorporation of this substituent did afford a very potent inhibitor (**2**),[10] although the corresponding unsubstituted analogue (**1**) also displayed excellent potency. Interestingly, although initially it appears that increasing the size of the substituent leads to a decrease in potency (cf **2** with **3** and **5**), incorporation of the ortho acetamido group (**6**) is well tolerated and may suggest that this group is able to participate in a hydrogen bonding interaction with the protein. Incorporation of a second methyl group at any of the other positions in the terminal ring other than the 6-position (**10**) is clearly disfavoured (**7**, **8**, and **9**), suggesting a tight binding pocket (Tables 1–4).

A feature of many urea containing VLA-4 antagonists is the presence of a methyl group beta to the carboxylate moiety.[12] Intrigued by this observation we decided to explore the SAR around this part of the molecule. Incorporation of the methyl group into the side chain of our inhibitors did afford a modest increase in potency in the *R*-enantiomer (cf **11** and **13**). Unfortunately, incorporation of the potency enhancing B-ring substituent[10] and a β-methyl moiety did not afford an additive increase in potency in the more potent *R*-enantiomer (cf **13** and **15**) but did afford a modest increase in the

Keywords: VLA-4; Integrin; Pyridone; Bioavailability.
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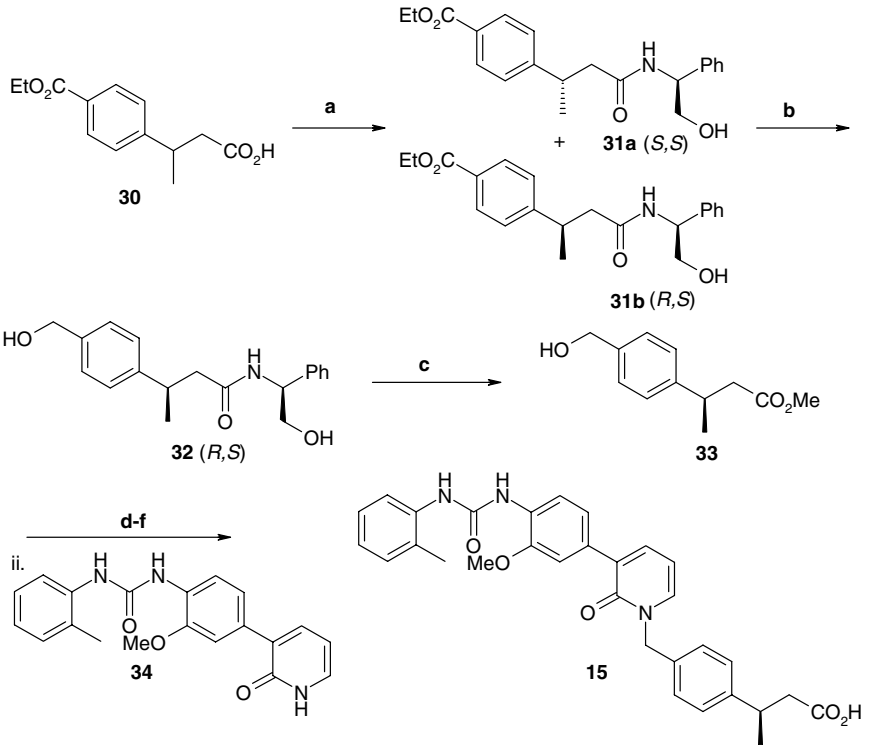
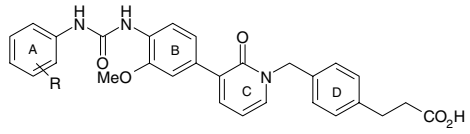
doi:10.1016/j.bmcl.2006.08.044



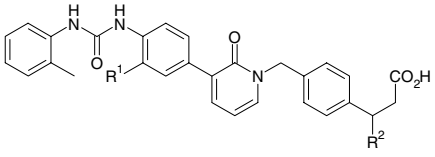
Scheme 1. Reagents and conditions: (a) i—$(COCl)_2$, 0 °C; ii—(2*S*)-2-amino-2-phenylethanol, Et_3N, DCM, 0–25 °C (94%); (b) $LiBH_4$, MeOH, THF (90%); (c) i—3 N H_2SO_4, dioxane, reflux (94%); ii—concd H_2SO_4, MeOH, reflux (69%); (d) MsCl, Et_3N, DCM (100%); (e) **34**, Cs_2CO_3, DMF, 25 °C (87%); (f) 0.5 N aq LiOH, THF, 25 °C (89%).

Table 1. A-ring SAR



Compound	R	pK_i α4β1[14]
1	H	8.8
2	2-Me	9.3
3	2-*i*Pr	6.5
4	2-OMe	8.2
5	2-Br	8.3
6	2-NHAc	8.2
7	2,3 DiMe	7.6
8	2,4 DiMe	7.6
9	2,5 DiMe	8.1
10	2,6 DiMe	8.7

Table 2. The β-methyl SAR



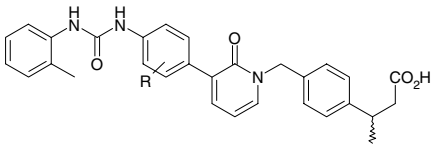
Compound	R^1	R^2	pK_i α4β1
2	MeO	H	9.3
11	H	H	8.6
12	H	(*S*)-Me	8.6
13	H	(*R*)-Me	9.0
14	MeO	(*S*)-Me	9.0
15	MeO	(*R*)-Me	9.1

S-enantiomer (cf **12** and **14**) although this modification did not display a potency enhancement relative to the baseline compound **2**.

Since we had observed a modest increase in potency by incorporation of an ortho methoxy group into the B-ring of the unbranched propionic acid series we decided to revaluate the SAR in the substituted series. Unfortunately, incorporation of a variety of ortho substituents in either enantiomeric form failed to improve the potency over the baseline compounds **12** and **13**. Interestingly, however, the enantiomers displayed divergent SAR at the meta position with several groups being well tolerated in the *R* series but poorly tolerated in the *S* series (cf **21** with **28** and **22** with **29**).

Having identified several potent inhibitors we sought to determine whether they were orally bioavailable in the rat. Initial evaluation of **2** and **11** suggested that like many other urea containing VLA-4 antagonists, oral absorption was likely to be an issue.[13] Gratifyingly, incorporation of the β-methyl substituent afforded a modest improvement in oral absorption (cf **13** with **11**) and further profiling of this compound revealed the compound to have low clearance and modest but variable bioavailability. The profile was further improved by incorporation of the B-ring methoxy group (**13** with

Table 3. B-ring SAR in the β-methyl series



Compound	R	*R*/*S*	pK_i α4β1
16	2-Cl	*R*	9.0
17	2-OCF_3	*R*	8.4
18	2-OEt	*R*	8.7
19	2-Et	*R*	8.2
20	2-Me	*R*	9.0
21	3-Cl	*R*	9.1
22	3-Me	*R*	8.6
23	2-Cl	*S*	8.7
24	2-OCF_3	*S*	7.7
25	2-OEt	*S*	8.3
26	2-Et	*S*	7.3
27	2-Me	*S*	8.6
28	3-Cl	*S*	7.6
29	3-Me	*S*	7.7

15) possibly due to the formation of an intra-molecular hydrogen bond with the acidic urea functionality. Compound **15** displayed the most encouraging PK profile (%Fpo = 49, 84, 166, 185) and although the group size was small ($n = 4$) the exposure and clearance appeared highly variable suggesting events such as recycling and transporter mediated absorption and/or elimination may be contributing to the high, but variable, bioavailability.

The single enantiomers of the pyridone analogue **15** were obtained[15] by treatment of the known acid[16] **30** with oxalyl chloride followed by coupling with commercially available (2*S*)-2-amino-2-phenylethanol to afford the diastereomers **31a** and **31b** which were readily separable using conventional column chromatography. Reduction of **31b** with lithium borohydride afforded the corresponding alcohol **32** which was hydrolysed to the corresponding acid then converted to the methyl ester **33**. Treatment of **33** with methane sulfonyl chloride afforded the corresponding mesylate which was then reacted with the known pyridone[10] **34** to afford the intermediate ester which was then hydrolysed with lithium hydroxide to afford the desired analogue **15** in good overall yield.

In summary, having previously successfully employed a pyridone nucleus as a bioisostere for an amide moiety, we have systematically identified the key pharmacophoric features responsible for high inhibitory potency. In common with the literature the SAR around the A-ring suggests an ortho methyl group is optimal as is the presence of an ortho methoxy moiety in the B-ring. Unfortunately incorporation of both these potency enhancing substituents into the more potent β-methyl propionic acid series does not lead to an additive increase in potency. In vivo profiling of two of the more potent inhibitors suggests oral bioavailability is achievable with this series.

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Table 4. Rat pharmacokinetic profiles of selected VLA-4 antagonists

Compound	DNAUC[a] (min kg/L)	F[b,c] (%)	Cl (iv) (mL/min/kg)	$t_{1/2}$ (h)	VSS (L/kg)
2	6 ± 0.5	n.d.	n.d.	n.d.	n.d.
11	3 ± 3	n.d.	n.d.	n.d.	n.d.
13	14 ± 4	22 ± 15	16 ± 7	1.3 ± 0.7	0.6 ± 0.1
15	154 ± 82	121 ± 72	9 ± 12	1.4 ± 0.8	0.6 ± 0.4

[a] Dose normalised area under the curve (0–8 h).
[b] Male Sprague–Dawley rats ($n = 3$–4, ±SD).
[c] Dose: iv infusion at 1 mg/kg; po at 3 mg/kg ($n = 3$–4, ±SD).

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