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Imidazopyridines as VLA-4 integrin antagonists

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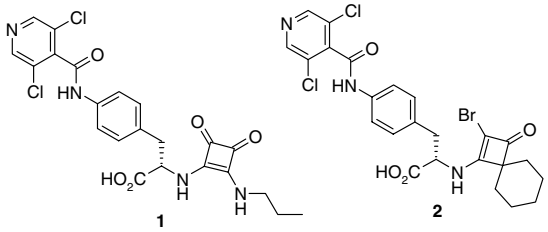
ABSTRACT

We describe a novel series of imidazopyridine substituted phenylalanines which are potent VLA-4 antagonists. A wide variety of substituents are tolerated as replacements for the pendant 3-pyridyl ring. A clear structure–activity relationship was identified around the substitution of the 3-amino-cyclobut-2-enone portion of the molecule.

The integrin α4β1 (VLA-4) is predominantly expressed on the cell surface of mononuclear leukocytes and eosinophils. VLA-4 is a heterodimeric adhesion molecule which binds to two receptors, fibronectin and vascular cell adhesion molecule 1 (VCAM-1) expressed on endothelial cells.[1] This binding interaction occurs in response to inflammatory cytokines and enables rolling, adhesion and transmigration of the cell through the endothelium to the site of inflammation.[2] Natalizumab (Tysabri®), is a therapeutic monoclonal IgG antibody currently on the market which has validated VLA-4 antagonism as a therapeutic approach for the treatment of inflammatory diseases such as multiple sclerosis[3] and Crohn's disease.[4]

Research into small molecule VLA-4 antagonists has been an active area in the pharmaceutical industry for over 10 years.[5] As a part of our ongoing research programme we have previously reported that compound **1** is a potent VLA-4 antagonist.[6] We have also shown that the cyclobutene 1,2-dione substituent can be replaced with functionalized 3-amino-cyclobut-2-en-1-ones as shown by compound **2**.[7] Our search for a new chemical series led to the discovery that replacement of the 4-pyridyl amide in compound **2** with various imidazopyridines gives rise to VLA-4 antagonists of comparable potency.

The compounds were readily prepared according to the methods outlined in Scheme 1. In method A, (*S*)-2-amino-3-(4-nitro-phenyl)-propionic acid ethyl ester hydrochloride **3** was heated in the presence of an appropriately substituted cyclobuta-1,3-dione[7] **4** to afford the vinylogous amide. The nitro group was reduced to the aniline **5** using palladium on carbon and hydrogen. This was then reacted with 2-chloro-3-nitropyridine or 4-chloro-3-nitropyridine **6**, displacing the labile chloride in refluxing ethanol to afford the nitropyridine. In the case of compound **9g**, aniline **5** was reacted with 2,5-difluoronitrobenzene. Hydrogenation of the nitro group gave the corresponding aromatic amine **7** which was then reacted with nicotinic acid using EDC and HOBT as coupling reagents to yield the amide **8**. Ring closure to form the imidazopyridine was achieved in acetic acid, with heating in a microwave reactor. Introduction of the halogen, when required, was carried out by reaction with *N*-bromosuccinimide (NBS) or *N*-chlorosuccinimide (NCS) in ethyl acetate. Hydrolysis in sodium hydroxide gave carboxylic acids **9a**–**g**.



1 **2**

In method B, (*S*)-3-(4-amino-phenyl)-2-*tert*-butoxycarbonylamino-propionic acid ethyl ester **10** was reacted with 2-chloro-3-nitropyridine to give the nitro compound **11**. The BOC protecting

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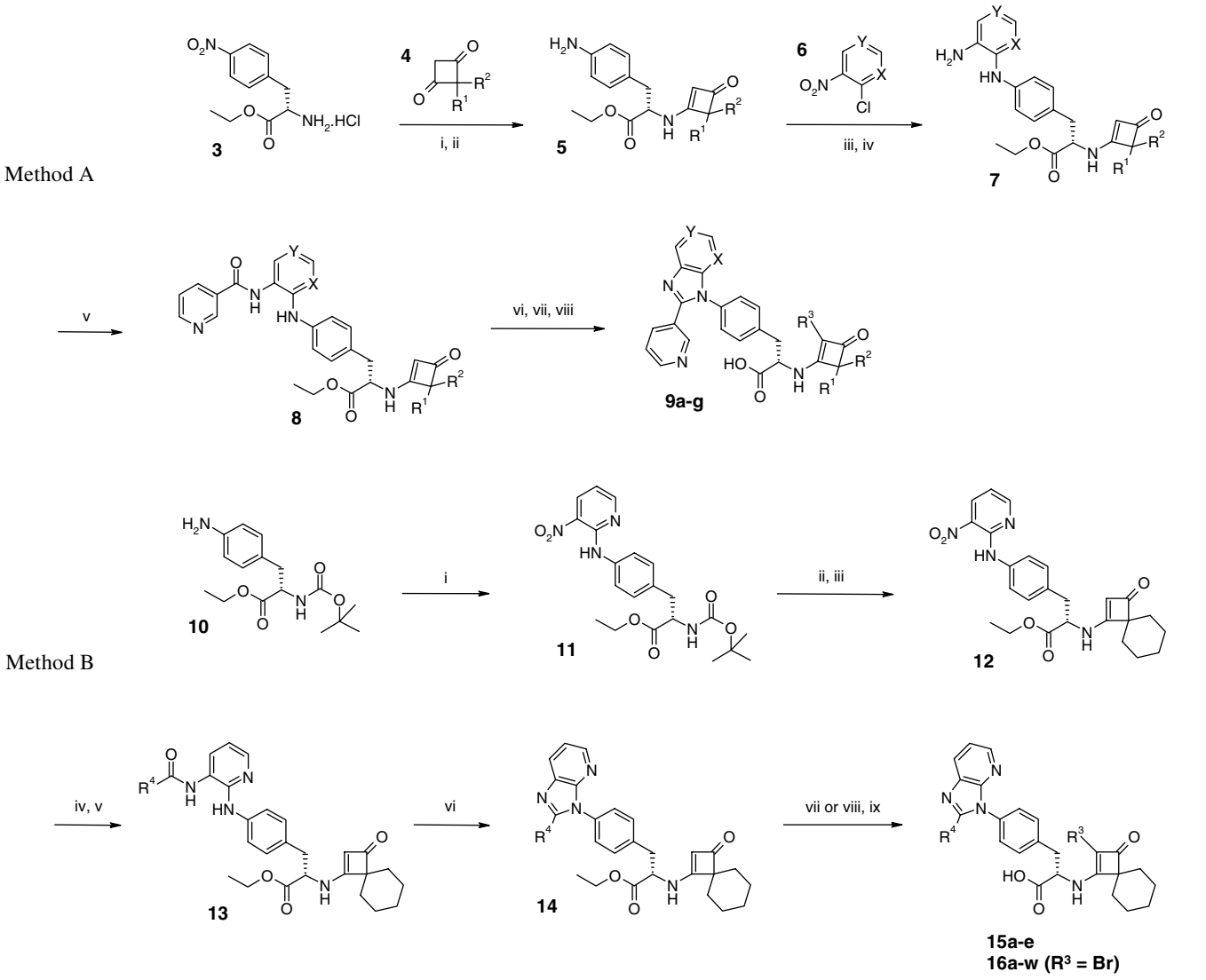


Scheme 1. Reagents and conditions: Method A (i) **4**, Et_3N, EtOAc, 80 °C, 18 h, 87–100%; (ii) H_2, Pd-C, EtOH, 100%; (iii) **6** or 2,5-difluoronitrobenzene, EtOH, DIPEA, reflux, 18–96 h, 16–100%; (iv) H_2, 10% Pd-C, EtOH, 100%; (v) nicotinic acid, EDC, HOBT, DCM, 89%; (vi) AcOH, microwave, 125 °C, 10 min, 75–100%; (vii) NBS or NCS, EtOAc, 38–100%; (viii) 2 M NaOH, THF, H_2O, 6–54%. Method B (i) 2-chloro-3-nitropyridine, EtOH, DIPEA, reflux, 18 h, 47%; (ii) TFA, DCM, 94%; (iii) spiro[3.5]nonane-1,3-dione, EtOAc, 80 °C, 18 h, 100%; (iv) H_2, Pd-C, EtOH, 100%; (v) R^4COOH, EDC, HOBT, DCM, 21–100%; (vi) AcOH, microwave, 125 °C, 10 min, 32–98%; (vii) NBS or NCS, EtOAc, 39–100%; (viii) $(iPrS)_2$ or $(MeS)_2$, SO_2Cl_2, THF, 53–55%; (ix) 2 M NaOH, THF, H_2O, 12–82%.

group was removed to yield the amine which was reacted with spiro[3.5]nonane-1,3-dione to furnish the vinylogous amide **12**. Reduction of the nitro group followed by reaction with a variety of carboxylic acids gave the amide **13**. Ring closure afforded the imidazopyridine **14**. Reaction with NBS or NCS when required followed by hydrolysis gave access to compounds **15a**–**c** and **16a**–**w**. Alternatively, treatment of compound **14** with a cooled premixed solution of the appropriate alkyl disulfide and sulfuryl chloride followed by hydrolysis furnished carboxylic acids **15d**,**e**.

Initially, we looked at the SAR around the 3-amino-cyclobut-2-enone portion of the molecule. Substitution was investigated at the spiro centre (R^1 and R^2) and at the double bond (R^3). When gem dimethyl groups were attached to the ring at the spiro centre we discovered an interesting trend. Compound **9b** (Table 1) with a chlorine atom at the double bond was approximately 6-fold more active in the whole blood assay[8] compared to the unsubstituted compound (**9a**). There was a further small increase with a bromine atom as the substituent (**9c**). When a cyclohexyl ring was attached at the spiro centre (**15a**) (Table 2) the whole blood activity was approximately 5-fold higher than gem dimethyl (**9a**). We observed

Table 1
Human whole blood assay data (IC_{50})

Compound	Method	R^1 and R^2	R^3	X	Y	Human whole blood[8] IC_{50} (nM)
9a	A	Me	H	N	CH	126
9b	A	Me	Cl	N	CH	19
9c	A	Me	Br	N	CH	10
9d	A	Cyclohexyl	H	CH	N	1616
9e	A	Cyclohexyl	Cl	CH	N	504
9f	A	Cyclohexyl	Br	CH	N	236
9g	A	Cyclohexyl	Br	CH	CF	2683

Table 2
Human whole blood assay data (IC_{50})

Compound	Method	R^1 and R^2	R^3	R^4	Human whole blood[8] IC_{50} (nM)
15a	B	Cyclohexyl	H	3-Pyridyl	24
15b	B	Cyclohexyl	Cl	3-Pyridyl	7
15c	B	Cyclohexyl	Br	3-Pyridyl	3
15d	B	Cyclohexyl	S-*i*-Pr	3-Pyridyl	2
15e	B	Cyclohexyl	SMe	3-Pyridyl	2

Table 3
Human whole blood assay data (IC_{50})

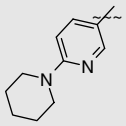
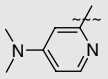
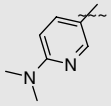
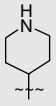
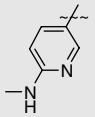
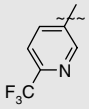
Compound	R^4	Human whole blood[8] IC_{50} (nM)
15c		3
16a		570
16b		95
16c		83
16d		46
16e		71
16f		22
16g		22
16h		18
16i		11
16j		14
16k		13
16l		11
16m		10
16n		10
16o		9
16p		9
16q		8
16r		8

Table 3 (*continued*)

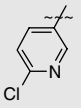
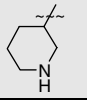
Compound	R^4	Human whole blood[8] IC_{50} (nM)
16s		3
16t		3
16u		2
16v		3
16w		6

Table 4
In vitro DMPK

Compound	Microsomes CLint (μL/min/mg) @ 0.5 μM			CYP inhibition IC_{50} (μM)			
	Human	Rat	Mouse	2D6	1A2	3A4	2C9
15c	<1	48	11	>100	>100	62	10
16o	4	38	14	>100	>100	79	18
16u	6	52	15	>100	>100	10	8

previously described with the gem dimethyl compounds. A bromine atom at R^3 (**15c**) was superior to chlorine (**15b**). The introduction of S-*i*-Pr (**15d**) or SMe (**15e**) at R^3 gave no significant improvement over a bromine atom (**15c**).

We also investigated changing the position of the nitrogen atom in the imidazopyridine ring (**9d**, **9e**, **9f**). This resulted in a marked loss of activity, however, the trend in double bond substitution continues across this series as described above. Removal of the nitrogen altogether (**9g**) also resulted in a significant loss of activity. As the presence and position of the nitrogen is key for activity we kept this fixed at position X in all further work.

In an attempt to find an alternative to the pyridine substituent attached to the imidazopyridine ring, more analogues were synthesized via Scheme 1, method B. This robust route allowed rapid access to a wide range of compounds with various R^4 substituents.

The data for these compounds are presented in Table 3 (R^3 was a bromine atom in all cases). The data show that a wide variety of groups are tolerated at R^4. We investigated substitution of the pyridine substituent of **15c** at the 6 position. These compounds (**16c**, **16g** and **16p**) were accessed by heating **16s** and the appropriate amine in a microwave using methanol or ethanol as the solvent. Introduction of a 6-amino substituent was found to be detrimental to activity. Substitution with NHMe (**16p**) reduced activity by 3-fold compared to the unsubstituted pyridine (**15c**). Larger amino substituents were not as well tolerated. For example, NMe_2 (**16g**) decreased activity by 2-fold compared to NHMe (**16p**). The larger piperidine (**16c**) was 9-fold less potent than NHMe (**16p**).

Alkyl substituents exhibited a range of activities. None of these brought any significant advantage over the pyridine of **15c**. The *tert*-butyl (**16t**) and trifluoromethyl cyclopropyl (**16u**) substituents had comparable activities to **15c** at 3 nM and 2 nM, respectively.

A simple phenyl substituent (**16e**) was not as effective as the pyridine while 2 and 3-piperidines exhibited comparable activities (**16v** and **16w**, respectively).

On the basis of excellent whole blood potency and chemical diversity we selected **15c**, **16o** and **16u** for drug metabolism and pharmacokinetics (DMPK) profiling (Table 4). Of these three R^4 substituents the isopropyl group (**16o**) had a slight advantage in terms of the cleanest cytochrome P450 (CYP) profile. Compound **16u** moderately inhibited both CYP3A4 and CYP2C9. Overall, **16o** had the best in vitro DMPK profile and a human whole blood potency of 9 nM.

Initial studies suggest that these compounds have an encouraging in vitro DMPK profile. However, there is evidence that biliary excretion is a characteristic of this compound class.[9] We are currently investigating this to determine the relationship between the in vitro DMPK and the in vivo clearance.

In conclusion, we have identified a novel and extremely potent series of VLA-4 antagonist. We have identified a clear SAR around the substitution of the 3-amino-cyclobut-2-enone portion of the molecule. Cyclohexyl substitution at the spiro centre coupled with bromine at the double bond was found to be optimal. It is clear that the position of the nitrogen in the imidazopyridine ring is important for activity and that removing it altogether is not tolerated. We have investigated over 20 substituents at position R^4 and found that many of these retain significant activity.

References and notes

1. (a) Elices, M. J. In *Cell Adhesion Molecules and Matrix Proteins: Role in Health and Diseases*; Mousa, S. A., Ed.; Springer and R.G. Landes Co: Berlin, 1998; p 133; (b) Elices, M. *J. Curr. Opin. Anti-inflamm. Immunomod. Invest. Drugs* **1999**, *1*, 14.
2. Kitayama, J.; Fuhlbridge, R. C.; Puri, K. D.; Springer, T. A. *J. Immunol.* **1997**, *159*, 3929.
3. Miller, D. H. et al *N. Eng. J. Med.* **2003**, *348*, 15.
4. Sandborn, W. J. et al *N. Eng. J. Med.* **2005**, *353*, 1912.
5. Davenport, R. J.; Munday, J. R. *Drug Discov. Today* **2007**, *12*, 569.
6. Porter, J. R.; Archibald, S. C.; Childs, K.; Critchley, D.; Head, J. C.; Linsley, J. M.; Parton, T. A.; Robinson, M. K.; Shock, A.; Taylor, R. J.; Warrellow, G. J.; Alexander, R. P.; Langham, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1051.
7. Brand, S.; de Candole, B. C.; Brown, J. A. *Org. Lett.* **2003**, *5*, 2343.
8. Compounds are diluted in TBS to 50 times desired start concentration. Compounds are titrated across a NUNC immunosorp plate (50 μL into 100 μL to give tripling dilutions). Ninety-five microlitres of blood are incubated with 3 mM $MnCl_2$ (Sigma—M-9522) and 1 μL streptavidin-FITC (Pierce—21224), with or without 10 μg/mL biotinylated dimeric hVCAM-mFc in FACS tubes (Falcon—352052). Two microlitres diluted compound are spiked into relevant tube and left for 30 min at room temperature on a shaking platform. Red blood cells are lysed with 2 mL FACS lysing solution (BD Biosciences—349202), and the remaining leukocytes are pelleted by centrifugation at 1200 rpm for 5 min. Leukocytes are washed twice with 3 mL TBS before resuspension in 100 μL TBS for analysis by flow cytometry. Values are the mean of at least two experiments.
9. (a) Porter, J. R.; Archibald, S. C.; Brown, J. A.; Childs, K.; Critchley, D.; Head, J. C.; Parton, T. A.; Robinson, M. K.; Shock, A.; Taylor, R. J.; Warrellow, G. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 805; (b) Tang, W.; Stearns, R. A.; Chen, Q.; Bleasby, K.; Teffera, Y.; Colletti, A.; Hafey, M.; Evers, R.; Dean, D. C.; Magriotis, P. A.; Lanza, T. J.; Lin, L. S.; Hagmann, W. K.; Baillie, T. A. *Xenobiotica* **2008**, *38*, 223.