Bioorganic & Medicinal Chemistry Letters 19 (2009) 5803–5806

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of *N*-{*N*-[(3-cyanobenzene) sulfonyl]-4(*R*)-(3,3-difluoropiperidin-1-yl)-(L)-prolyl}-4-[(3′,5′-dichloro-isonicotinoyl) amino]-(L)-phenylalanine (MK-0617), a highly potent and orally active VLA-4 antagonist

Shankar Venkatraman [a,*], Alec D. Lebsack [a,†], Kenneth Alves [c], Michael F. Gardner [b,‡], Joyce James [b,‡], Russell B. Lingham [c], Salony Maniar [c], Richard A. Mumford [c], Qian Si [c], Nicholas Stock [a,§], Kelly M. Treonze [c], Bowei Wang [a,§], Jasmine Zunic [a,§], Benito Munoz [a,¶]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, USA
[b] Department of Drug Metabolism, Merck Research Laboratories, San Diego, CA 92121, USA
[c] Department of Inflammation and Rheumatology Research, Merck Research Laboratories, Rahway, NJ 07065, USA

ARTICLE INFO

Article history:
Received 9 April 2009
Revised 14 July 2009
Accepted 15 July 2009
Available online 28 July 2009

Keywords:
VLA-4
Antagonist
Multiple scelerosis

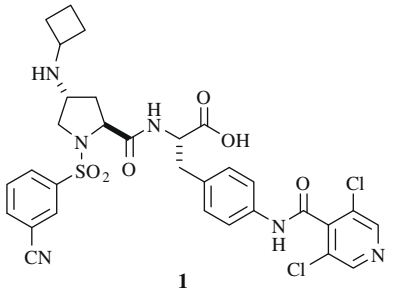
ABSTRACT

A series of prolyl-*N*-isonicotinoyl-(*L*)-4-aminophenylalanine derivatives substituted at the proline 4-position with cyclic amines was evaluated as VLA-4 antagonists. The ring size and presence or absence of fluorine affected potency and receptor occupancy. The analog with 3,3-difluoropiperidine at the proline 4-position (**13**) was the most potent compound and had very good duration of receptor occupancy in vitro. The ethyl ester prodrug of **13** demonstrated excellent receptor occupancy after oral dosing in rats.

The integrin very late antigen-4 (VLA-4, $\alpha_4\beta_1$) belongs to the heterodimeric integrin receptor superfamily.[1] VLA-4 is expressed on the surface of most leukocytes and binds to vascular cell adhesion molecule-1 (VCAM-1) and the CS-1 domain of fibronectin. VCAM-1 is expressed on activated endothelial cells in response to inflammatory cytokines. Adhesion interactions between VLA-4 and VCAM-1 may be important for activation, migration, proliferation, and differentiation of leukocytes during normal and pathophysiological processes.[2] Tysabri, a humanized monoclonal antibody against VLA-4, is approved for patients suffering from multiple sclerosis and Crohn's disease.[3] This has generated significant interest in the development of small molecule antagonists of VLA-4 for the treatment of diseases where cell-trafficking and activation are important, such as asthma, rheumatoid arthritis and multiple sclerosis.[4]

In our laboratory, earlier work on VLA-4 antagonists identified potent prolyl-dipeptides containing secondary amines with slow relative off-rates such as **1** (IC_{50} = 0.05 nM; receptor occupancy = 64% bound @ 3 h, Fig. 1).[5] The lead compound **1** did not achieve desirable levels of receptor occupancy in vivo. Herein, we report the synthesis and SAR of analogs of **1** substituted with cyclic tertiary amines at the proline 4-position as a means to obtain improved potency and in vivo properties.

Inhibition potency was measured using a competition binding assay with a ^{35}S labeled ligand and VLA-4 in its non-activated state



1

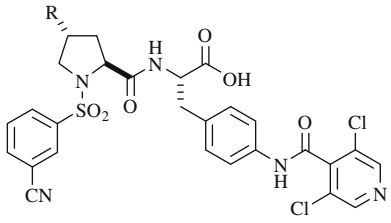
$\alpha_4\beta_1$ (Ca^{2+}, Mg^{2+}) IC_{50} = 0.05 nM
Receptor occupancy (Ca^{2+}, Mg^{2+}) =
64%bound @ 3 h

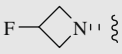
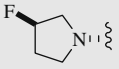
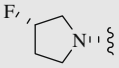
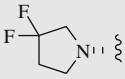
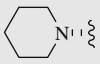
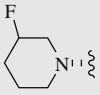
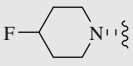
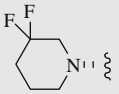
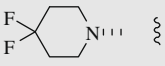
Figure 1. Lead compound for the program.

* Corresponding author. Tel.: +1 2153532900.
E-mail address: p3ptide@yahoo.com (S. Venkatraman).
† Present address: J& J San Diego, CA, United States.
‡ Present address: Ambit Biosciences, San Diego, CA, United States.
§ Present address: Amira Pharmaceuticals, San Diego, CA, United States.
¶ Present address: Broad Institute, Cambridge, MA, United States.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.07.111

Table 1
Binding to VLA-4 by 4-substituted prolyl-*N*-isonicotinoyl-(L)-4-aminophenylalanine derivatives



Compds[a]	R	$\alpha_4\beta_1$ Ca^{2+}, Mg^{2+} IC_{50} (nM)	In vitro relative off-rate (37 °C) @ 3 h (% bound @ 3 h)
1		0.15 ± 0.01	64 ± 10
2		0.02 ± 0.02	80 ± 12
3		0.15 ± 0.03	56 ± 6
4		0.27 ± 0.06	60±6
5		0.90 ± 0.12	65
6		0.22 ± 0.07	65
(*R*)-**7**		0.12 ± 0.03	80
(*S*)-**8**		0.24 ± 0.07	75
9		0.10 ± 0.03	81 ± 12
10		0.11 ± 0.03	80 ± 8
11[b]		0.21 ± 0.06	70
12		0.35 ± 0.13	55
13		0.03 ± 0.01	90 ± 6
14		0.22 ± 0.05	37 ± 4

[a] All data is an average of three independent runs or $n = 1$.
[b] Mixture of diastereomers.

(Ca^{2+}, Mg^{2+} buffer) in Jurkat cells.[6] In addition, competitive binding assay in whole blood and dissociation kinetics expressed as % bound after three hours were utilized to further distinguish these compounds.[7–9] As shown in Table 1, a variety of fluorinated cyclic tertiary amine-dipeptide derivatives were evaluated using these assays in tandem.

The structure–activity trends in Table 1 suggest that both ring size and presence or absence of fluorine at various positions are important in optimizing potency and receptor occupancy. Increasing the ring size from 4 through 6 provided greater receptor occupancy; optimal results were obtained with piperdines **10** and **13**. In general, 3,3-difluoroazacycles **5**, **9**, and **13** were more potent (IC_{50} = 0.03–0.10 nM) and afforded higher receptor occupancy (70–90% bound @ 3 h) than the corresponding des-fluorine analogues **3**, **6**, and **10**. Additionally, most compounds presented in Table 1 showed higher levels of receptor occupancy (>58% bound @ 3 h) when compared with the initial cyclobutyl amine lead **1**. The 3,3-difluoropiperdine **13** afforded the best combination of binding affinity (IC_{50} = 0.03 nM) and receptor occupancy (87% bound @ 3 h).

Interestingly, 4,4-difluoropiperdine **14** was sevenfold less potent and exhibited a 2.5-fold lower receptor occupancy than 3,3-difluoropiperdine **13**, suggesting that location of fluorine groups is critical. Only small differences in potency and receptor occupancy could be observed with enantiomers of mono-fluorinated compounds such as **7** and **8**. Differences in the pK_a values of fluorinated verses des-fluorinated cyclic tertiary amines did not explain the differences in binding affinities and receptor occupancy. For example, **10** and **13** exhibit nearly identical binding affinities and receptor occupancies, while their measured pK_a values for the amine were 8.4 and 4.6, respectively.[10] Introduction of 2,2,2-trifluoroethyl in the lead compound provided **2** with improved potency, but **2** exhibited a significant shift to lower potency in the human whole blood assay, (IC_{50} = 3.1 nM) compared to cyclic amines such as **13** which exhibited little shift in the same assay (IC_{50} = 0.07 nM).

Since monoclonal antibodies have the ability to recognize both the activated and non-activated states of VLA-4,[2] we explored the binding and receptor occupancy for 3,3-difluoropiperdine **13** using the activated state of VLA-4 (Mn^{2+}, Fig. 2). The potency of 3,3-difluoropiperdine **13** with the activated form of VLA-4 afforded an IC_{50} value of 0.02 nM, similar to that of the resting state (IC_{50} = 0.03 nM). In addition, **13** exhibited similar receptor occupancies at 3 h using the activated and non-activated forms of VLA-4. Interestingly, when the receptor occupancies at 3 h were compared for **13** and the initial lead **1**, we could only differentiate the two compounds using the non-activated state of VLA-4. The results demonstrated utility of the receptor occupancy assay to better distinguish between compounds in the non-activated state and highlighted the improved receptor occupancy of 3,3-difluoropiperdine **13** compared to **1**.

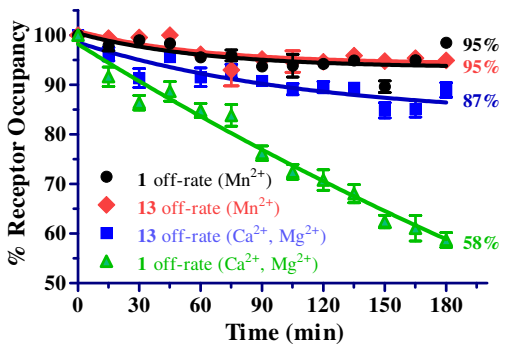


Figure 2. Receptor occupancy versus time curves for **1** and **13** under activating (Mn^{2+}) and non-activating (Ca^{2+}, Mg^{2+}) conditions.

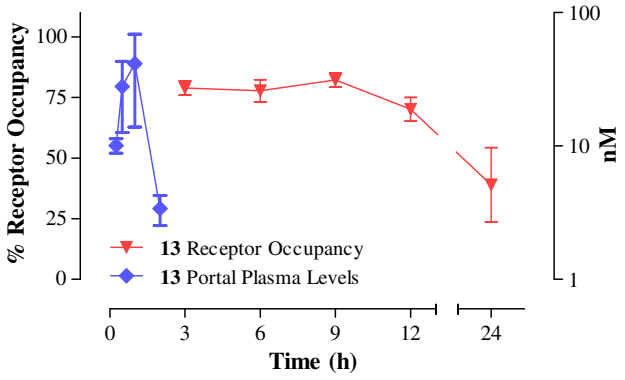


Figure 3. Portal plasma levels and VLA-4 receptor occupancy in rats following oral dosing of **13** at 5 mpk.

As a prelude to evaluating 3,3-difluoropiperdine **13** in animal models of autoimmune and allergic diseases, the receptor occupancy and pharmacokinetics were measured (Fig. 3). While the systemic pharmacokinetics were poor for **13** (AUC and C_{max} could not be determined; Cl_p >100 mL/min/kg; F <5%), portal plasma levels provided an AUC of 50 nM h and a C_{max} of 27 nM. Although the drug levels were low, the C_{max} was 75-fold over the average concentration of VLA-4 receptors in the rat (300 pM). Furthermore, in the receptor occupancy assay, **13** achieved 70% occupancy at 12 h and 40% occupancy at 24 h (Fig. 2). The initial exposure of **13** to circulating leukocytes bearing VLA-4 in the portal vein and slow off-rate likely compensate for the poor systemic pharmacokinetics to provide receptor occupancy coverage even after the drug levels can no longer be detected. The correlation between receptor occupancy and efficacy in animal models and human is well documented.[8]

Furthermore, when **13** was dosed as the ethyl ester prodrug at 1.5 mpk in rats, it yielded receptor occupancy values of 88% and 60% at 12 h and 24 h, respectively. This is in contrast to the parent acid which, when dosed at 5 mpk orally in rats, gave receptor occupancy values of 65% and 22% at 12 h and 24 h, respectively. The ethyl ester prodrug of **13** also had higher receptor occupancy compared to the ethyl ester prodrug of **1** at 1.5 mpk over 24 h as shown in Figure 4.

The fluorinated and non-fluorinated cyclic tertiary amine-dipeptide derivatives were prepared as outlined in Scheme 1. The synthesis began with commercially available *cis*-4-hydroxy-L-proline methyl ester (**15**) which was sulfonylated with 3-cyano-sulfonyl-chloride (**16**). Exposure of the resulting sulfonamide to triflic anhydride (Tf_2O) in the presence of diisopropylethyl amine at −60 °C

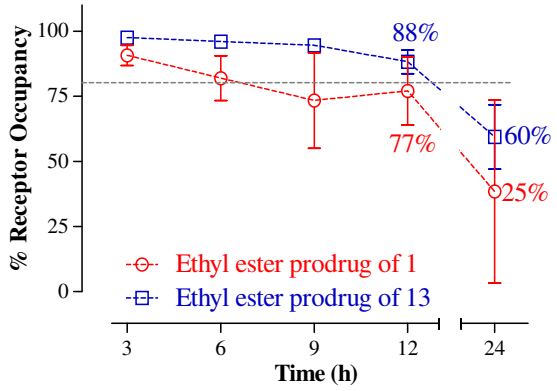
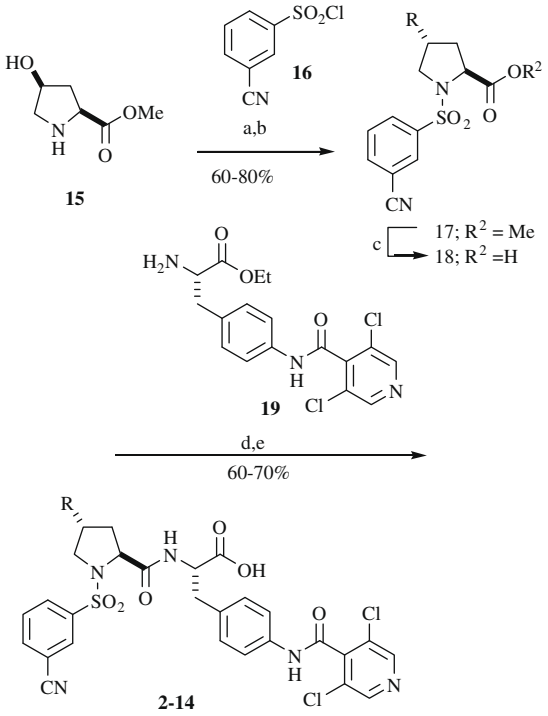


Figure 4. Rat Receptor occupancy of **1** and **13** following oral dosing of ester prodrugs at 1.5 mpk.



Scheme 1. Reagents and conditions: (a) **15**, Et_3N, CH_2Cl_2; (b) TfO_2, *i*PrEtN, CH_2Cl_2, −60 °C, 2 h then R, *i*PrEtN −20 °C to rt, 12 h; (c) LiOH, CH_3CN/H_2O; (d) **18**, EDC, HOBT, Et3 N, DMF; (e) LiOH, CH_3CN/H_2O.

provided a triflate. This was displaced with a variety of commercially available cyclic tertiary amines (*R*) to provide **17** in one pot.[9] Hydrolysis of the methyl ester **17** by reaction with lithium hydroxide afforded the corresponding acids **18**. Standard peptide coupling of these acids **18** with *N*-isonicotinoyl-(L)-4-aminophenylalanine (**19**) using 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole hydrate (HOBT) followed by hydrolysis of the ethyl ester in the presence of lithium hydroxide delivered the final acids **2–14**.[11]

In summary, we have developed a novel series of potent prolyl dipeptide VLA-4 antagonists containing fluorinated cyclic tertiary amines at the proline 4-position. In general, the fluorinated cyclic tertiary amines provided improved potency and receptor occupancy levels when compared with their des-fluoro analogues and the initial cyclobutyl amine lead compound. An optimal compound from this work is 3,3-difluoropiperdine **13 (MK-0617)** with an IC_{50} value of 0.03 nM and a receptor occupancy on both activated and non-activated VLA-4 receptors of ~90% at 3 h. Dosed orally as its ethyl ester prodrug to rats, **13** had low systemic exposure but excellent receptor occupancy at 24 h due to its slow off-rate.

Acknowledgments

We acknowledge the contributions of many members of the labeled synthesis group and the Laboratory of Animal Resources of Merck Research Laboratories.

References and notes

1. Hemler, M. E.; Elices, M. J.; Parker, C.; Takada, Y. *Immunol. Rev.* **1990**, *114*, 45.
2. Elices, M. J. *Curr. Opin. Anti-infamm. Immunol.* **1999**, *1*, 15.
3. (a) Kent, S. J.; Karlik, S. J.; Cannon, C.; Hines, D. K.; Yednock, T. A.; Fritz, L. C.; Horner, H. C. *J. Neuroimmunol.* **1995**, *58*, 1; (b) Honey, K. *J. Clin. Invest.* **2008**, *118*, 825.
4. For Asthma see: (a) Sielszak, M. W.; Ahmed, A.; Cortes, A.; Lauredo, I. T.; Kim, J.; Pepinsky, B.; Beenjamin, C. D.; Leone, D. R.; Lobb, R. R.; Weller,

P. F. *J. Clin. Invest.* **1994**, *93*, 776; (b) Milne, A. A. Y.; Piper, P. J. *Eur. J. Pharmacol.* **1995**, *282*, 243; For Rheumatoid Arthritis see: (c) Seiffge, D. *J. Rheumatol.* **1996**, *23*, 2086; For multiple sclerosis see: (d) Yednock, T. A.; Cannon, D.; Fritz, L. C. *Nature* **1992**, *356*, 63; (e) Keszthelyi, E.; Karlik, S.; Hyduk, S.; Rice, A.; Gordon, G.; Yednock, T.; Horner, H. *Neurology* **1996**, *47*, 1053.

5. Lin, L. S.; Lanza, T.; Jewell, J. P.; Liu, P.; Jones, C.; Kieczykowski, G. R.; Treonze, K.; Si, Q.; Manior, S.; Koo, G.; Tong, X.; Wang, J.; Schuelke, A.; Pivnichny, J.; Wang, R.; Raab, C.; Vincent, S.; Davies, P.; MacCoss, M.; Mumford, R. A.; Hagmann, W. K. *J. Med. Chem.* **2009**, *52*, 3449.
6. (a) Hagmann, W. *Curr. Top. Med. Chem.* **2004**, 4, 1461; (b) Hagmann, William K.; Lin, L. S.; Liu, P.; Mumford, R. A.; Reger, T. S.; Smith, N. D.; Stock, N. S.; Zunic, J. PCT Int. Appl. WO 2005087760 A1, 2008.; (c) For kinetic off rate measurements, Jurkat cells were first treated with saturating levels of compound at 37 °C. After one hour incubation, the cells were collected by filtration and washed once with ice-cold binding buffer. Subsequently, cells were re-suspended in the binding buffer containing an excess of [125*I*]-tracer, and further incubated for increasing periods of time. The cells were collected at different time points, and the radioactivity associated with the cells was measured in a Packard TopCount NXTTM.
7. Hagmann, W. K. *J. Med. Chem.* **2008**, *51*, 4359.
8. Sandborn, W. J.; Colombel, J. F.; Enns, R.; Feagan, B. G.; Hanauer, S. B.; Lawrance, I. C.; Panaccione, R.; Sanders, M.; Schreiber, S.; Targan, S.; Deventer, S. V.; Goldblum, R.; Despain, D.; Hogge, G. S.; Rutgeerts, R. *NEJM* **2005**, *353*, 1912.
9. (a) Doherty, G. A.; Yang, G. X.; Borges, E.; Tong, S.; McCauley, E. D.; Treonze, K. M.; Riper, G. V.; Pacholok, S.; Si, Q.; Koo, G. C.; Shah, K.; Mumford, R. A.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1891; (b) See Ref. 6a.; (c) Leone, D. R.; Giza, K.; Gill, A.; Dolinski, B. M.; Yang, W.; Perper, S.; Scott, D. M.; Lee, W.-C.; Cornebise, M.; Wortham, K.; Nickerson-Nutter, C.; Chen, L. L.; LePage, D.; Spell, J. C.; Whalley, E. T.; Petter, R. C.; Adams, S. P.; Lobb, R. R.; Pepinsky, R. B. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 1150.
10. HPLC log *D* also did not account for differences in binding affinity (all had similar values of 2 at pH 7.3).
11. All the final compounds were greater than 95% ee as measured by chiral HPLC using analytical column (CHIRALCEL OD 10 μ, 4.6 × 250 mm) with heptane and isoproponol/triethylamine as mobile system.