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Arylsulfonamide pyrimidines as VLA-4 antagonists

Ying-zi Xu [a,*], Andrei W. Konradi [a], Frederique Bard [b], Michael Dappen [a], Lilibeth Dofiles [c], Mark Dreyer [d], Ian Gallager [b], Caroline Garrido [b], Mike Krimm [c], Zhenmei Liao [c], Elizabeth Messersmith [c], Linda Mutter [d], Michael A. Pleiss [a], Bhushan Samant [d], Christopher M. Semko [a], Jennifer Smith [a], Frank Stappenbeck [a], Brian Stupi [a], Chris Vandervert [b], Brent Welch [b], Brian Wipke [c], Ted Yednock [b]

[a] Department of Chemical Sciences, Elan Pharmaceuticals, Inc., 180 Oyster Point Blvd., South San Francisco, CA 94080, USA
[b] Department of Biology, Elan Pharmaceuticals, Inc., 180 Oyster Point Blvd., South San Francisco, CA 94080, USA
[c] Department of Pharmacology, Elan Pharmaceuticals, Inc., 180 Oyster Point Blvd., South San Francisco, CA 94080, USA
[d] Department of DMPK, Elan Pharmaceuticals, Inc., 180 Oyster Point Blvd., South San Francisco, CA 94080, USA

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ABSTRACT

A series of (*S*)-2-(2-(diethylamino)-5-(*N*-alkyl-*N*-sulfonamido)pyrimidin-4-ylamino)-3-(4-(carbamoyloxy)phenyl)propanoic acid is discovered as orally available VLA-4 antagonists. Representative compounds **11b** and **11p** showed efficacy in multiple in vivo animal models. The in vitro selectivity of **11p** is also described.

Very late antigen 4 (VLA-4, α4β1 integrin) is an adhesion molecule, which is expressed on T-cells, B-cells, eosionophils, basophils and mast cells.[1] VLA-4 binds to vascular cell adhesion molecule 1 (VCAM-1), which is transiently expressed on endothelial cells in response to cytokines, and to fibronectin (FN), which is present in the extracellular matrix.[2] Interactions of VLA-4 with these ligands mediate the adhesion, extravascularization, and survival of lymphocytes, in certain beneficial or pathological states of inflammation. The humanized monoclonal antibody Natalizumab blocks the binding of VLA-4 to VCAM-1, and is an effective intravenous therapy for multiple sclerosis (MS).[3,4] Towards oral therapies for MS and other inflammatory diseases, considerable research has been devoted to the discovery of orally available small molecules, which block the function of VLA-4.[5–12] Dipeptide derivatives including **1** (Fig. 1) are very potent VLA-4 inhibitor as demonstrated by FN cell adhesion assay[13] but with poor oral availabilities, which have been reported by several research groups.[14] During the course of our VLA-4 inhibitor discovery program, we obtained the X-ray crystal structure of the dicyclohexylammonium salt of the moderately potent VLA-4 inhibitor Ts-Pro-Phe-OH **2**. The unit cell contained four very similar conformations of **2**, one of which is illustrated in Figure 1. In all four conformations, the proline residue was in a gamma-turn conformation, with a Psi angle near zero.

Given the observed proline gamma turn conformation, we hypothesized a 5-substituted pyrimidine might be an effective replacement for the amide bond in **1**. Applying the methods illustrated in Scheme 1, we converted the previously reported intermediate **4** into **3**, which proved to be a very potent VLA-4 inhibitor, albeit still with suboptimal oral availability.

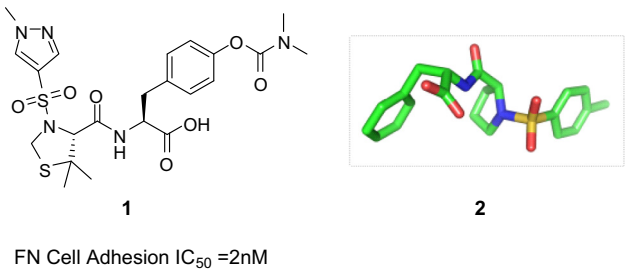

Figure 1. Very potent dipeptide derivative VLA-4 antagonist (**1**); Ts-Pro-Phe-OH (**2**) dicyclohexylammonium salt X-ray crystal structure (dicyclohexylammonium ion omitted for clarity).

* Corresponding author. Tel.: +1 6508570520.
E-mail address: ying_zi_xu@yahoo.com (Y.-z. Xu).

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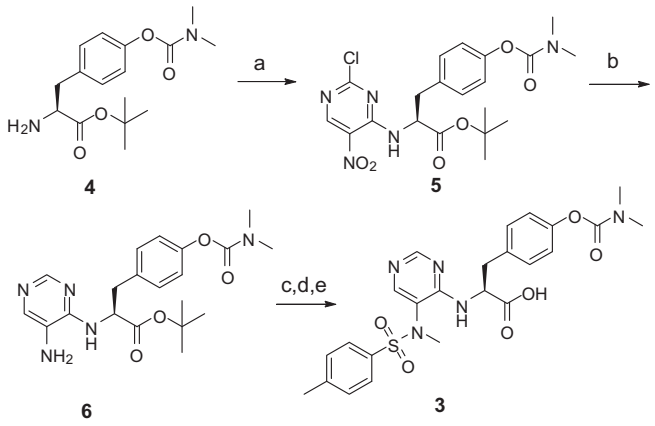
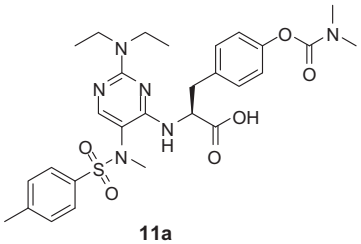


Scheme 1. Reagents and conditions: (a) 2,4-chloro-5-nitropyrimidine, *i*PrN(Et)$_2$, CH_2Cl_2, 0 °C; (b) Pd/C, H_2, $NaHCO_3$, EtOH; (c) TsCl, pyridine; (d) Me_2SO_4, K_2CO_3, DMF (e) HCO_2H.

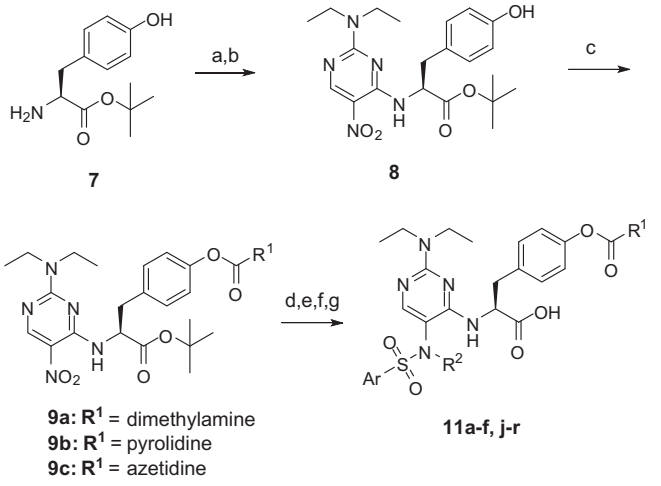
Previously, we reported that similar pyrimidines, with Et_2N groups attached on their 2-positions, are orally available VLA-4 inhibitors.[9] Applying the methods illustrated in Scheme 2, we prepared **11a** (Fig. 2), which has in vitro potency comparable to **3**, but 15% oral availability in rat.



Given the success of **11a**, we applied the methods illustrated in Schemes 2 and Scheme 3 to prepare a panel of pyrimidines, structures and data for which are illustrated in Table 1. All the products were determined to be very potent in our FN cell adhesion assay. However, there were strong substituent effects on systemic exposure as measured by cassette dosing.[15] In general, the best exposures were obtained for 4-fluorophenyl sulfonamides, with unbranched N-substituents, and carbamates derived from pyrrolidine. Compound **11p**, which combines an *N*-propargyl 4-fluorophenyl sulfonamide with a pyrrolidine carbamate, achieves very impressive exposure, as measured by either cassette dosing or a 12-h individual PK experiment.

Compounds **11b** and **11p** also were tested in FN cell adhesion assays including serum from humans, mice, or rats (Table 2). The IC_{50}s determined in the different sera suggest very strong binding of these compounds to proteins in human and rat sera, but much weaker binding to proteins in mouse serum.

A 0.3 mg/kg oral dose of compound **11b** was determined to inhibit the early and late phases of airway hyper-responsiveness and subsequent carbachol sensitivity in the Abraham sheep model of asthma[16] (data not shown). Compounds **11b** and **11p** were tested in a mouse model of acute pulmonary cellular influx.[17] Female Black/6 mice were sensitized on day 1 by intraperitoneal injection (i.p.) of ova/alum followed by a booster injection on day 14. Mice were challenged on day 28 and 29 with aerosolized ova and alum. Mice were euthanized and bronchoalveolar lavage samples were



Scheme 2. Reagents: (a) 2,4-dichloro-5-nitropyrimidine, *i*PrN(Et)$_2$, THF; (b) Et_2NH, *i*PrOH; (c) R^1COCl, Et_3N, CH_2Cl_2; (d) Pd/C, H_2, EtOH; (e) $ArSO_2Cl$, pyridine; (f) Me_2SO_4 or EtI or propargyl chloride, K_2CO_3, DMF; (g) HCO_2H

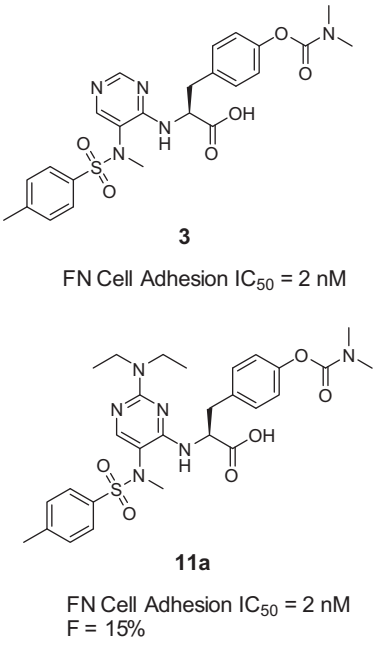
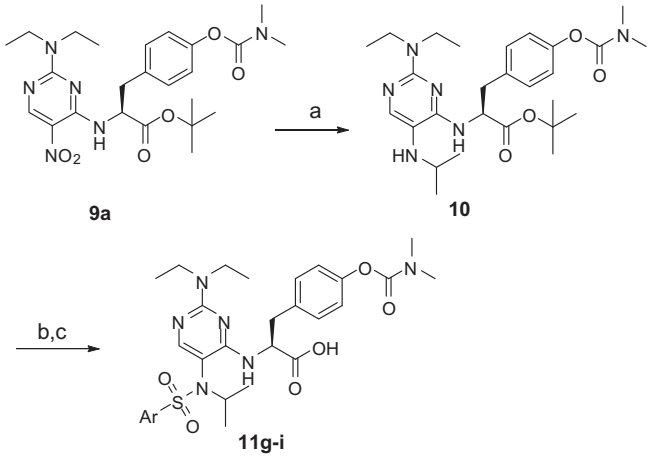


Figure 2. 5-Substituted pyrimidine as a proline replacement.



Scheme 3. Reagents: (a) acetone, AcOH, PtO_2, H_2, EtOH; (b) $ArSO_2Cl$, pyridine; (c) HCO_2H.

Table 1
In vitro potencies and exposures/pharmacokinetics in SD rat for compounds **11a–11r**

	Ar	R^1	R^2	FN Cell adhesion[a] IC_{50} (μM)	Cassette AUC (μg h/mL)	AUC iv (μg h/mL)	iv $t_{1/2}$	AUC po (μg h/mL)	C_{12} po[b] (μg/mL)	F[c] (%)
11a	p-Tolyl	NMe_2	Methyl	0.003		41	1.6	6	0.10 $_{(6h)}$	15
11b	4-Fluorophenyl	NMe_2	Methyl	0.002	1.78	27	4.1	20	0.12	74
11c	4-Chlorophenyl	NMe_2	Methyl	0.003	1.64	32	7.1	23	0.13	73
11d	4-Fluorophenyl	NMe_2	Ethyl	0.001	1.9	32	3.3	14	0.06	44
11e	4-Chlorophenyl	NMe_2	Ethyl	0.007	1.25	17	4.2	5.1	0.012	33
11f	3,4-Difluorophenyl	NMe_2	ethyl	0.004	0.55	17	3.6	4.2	0.008	24
11g	4-Fluorophenyl	NMe_2	*i*-Propyl	0.005	0.51					
11h	4-Chorophenyl	NMe_2	*i*-Propyl	0.004	0.43					
11i	3,4-Difluorophenyl	NMe_2	*i*-Propyl	0.007	0.12					
11j	p-Tolyl	Pyrrolidine	Methyl	0.006	20.54	173	6.1	74	0.45	43
11k	4-Chlorophenyl	Pyrrolidine	Methyl	0.019	13.2	153	10.2	51	0.33	33
11l	2,4-Difluorophenyl	pyrrolidine	Methyl	0.008	14.8	90	11.3	16	0.1	17
11m	4-Fluorophenyl	Pyrrolidine	Ethyl	0.001	12.8	111	5.9	51	0.18	46
11n	4-Chlorophenyl	Pyrrolidine	Ethyl	0.017	9.7	110	5.7	34	0.1	31
11o	2,4-Difluorophenyl	Pyrrolidine	Ethyl	0.009	4.8	83	11.1	28	0.05	34
11p	4-Fluorophenyl	Pyrrolidine	Propargyl	0.008	90.3	156	10.4	88	1.31	56
11q	2,4-Difluorophenyl	Pyrrolidine	Propargyl	0.013	23.8	126	9.2	42	0.12	33
11r	4-Fluorophenyl	Azetidine	Ethyl	0.002	0.9	34	6.9	6	0.01	16

[a] Fibronectin mediated cell adhesion assay.
[b] Compound level at 12 h post po dosing.
[c] F = bioavailability.

Table 2
IC_{50}s of **11b** and **11p** in FN cell adhesion assays with and without sera from human, rat, and mouse

	α4β1-FN IC_{50} (μM)			
	0.3% BSA	100% Human serum	100% Rat serum	100% Mouse serum
11b	0.002	0.045	0.28	0.006
11p	0.009	4.6	1.9	0.036

collected on day 30, 48 h post first challenge. The α_4 antibody, PS/2, was administered iv at 10 mg/kg, 2 h prior to the first challenge. Compounds **11b** and **11p** were administered twice daily on days 28 and 29 by oral gavage, at a constant dose volume of 5 ml/kg, 1 h prior and 12 h post challenge. The typical range of inhibition achieved by PS/2 was 60–80%. As illustrated in Table 3, **11b** and **11p** had minimum effective po doses of 10 and 3 mg/kg, respectively.

The robust efficacy of **11p** in the mouse model of pulmonary cellular influx prompted us to test this compound in a rat collagen induced arthritis (CIA) model.[18,19] Female Lewis rats with developing type II collagen arthritis were treated prophylactically by oral gavage, bid, with 3, 10 and 30 mg/kg **11p** for 21 days. In a separate arm of the study, similar animals were given methotrexate (MTX) 0.05 mg/kg po, qd alone or in combination with **11p**. Efficacy evaluation was based on ankle caliper measurements, expressed as area under the curve (AUC), terminal hind paw weights and

Table 3
Efficacy of **11b** and**11p** in a mouse model of acute pulmonary cellular influx

	11b		**11p**	
	10 mg/kg	3 mg/kg	3 mg/kg	1 mg/kg
Eos influx% inhibition wrt [a] PS/2[b]	80**	5	74*	0
Eos influx% inhibition wrt [a] vehicle group	45**	3	41*	0

[a] With respect to.
[b] PS/2 is an α_4 antibody, it was used as positive control in the study. The typical range of inhibition achieved was 60–80%.
** p <0.01.
* p <0.05.

Table 4
Efficacy of **11p** in the rat CIA model

	11p			**11p** + MTX 0.05 mg/kg			MTX
	30 mg/kg	10 mg/kg	3 mg/kg	30 mg/kg	10 mg/kg	3 mg/kg	0.05 mg/kg
AUC paw diameter	37**	31*	25	41**	24*	21	24
Final paw weight	23*	26*	15	37**	11	25*	17
Body weight	15	14	0	26*	27*	13	28*
Ankle histology	26**	26*	24*	31**	23*	21*	17
Knee histology	37**	40**	39**	46**	46*	17	31*

** p <0.01.
* p <0.05.

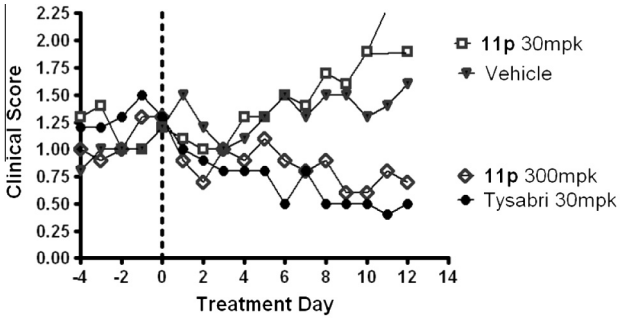


Figure 3. EAE experiment of **11p** with Tysabri as positive control.

Table 5
Activity of **11p** in adhesion assays involving different integrins

Assays	IC_{50} (μM)
$\alpha_4\beta_1$-FN	0.009
$\alpha_4\beta_7$-MadCAM	7.24
$\alpha_L\beta_2$-ICAM	>30
$\alpha_v\beta_1$-OPN	0.5
$\alpha_5\beta_1$-FN	>30

histopathologic evaluation of ankles and knees. Results were expressed using only animals that had definite clinical and histopathologic evidence of disease induction that resulted in connective tissue damage. As illustrated in Table 4, **11p** had significant effects on inflammation, cartilage destruction, and bone resorbtion. Rats treated with **11p** at 30 mg/kg or 10 mg/kg alone and in combination with MTX had significant inhibition of clinical arthritis as assessed by AUC for ankle swelling. Rats given **11p** at 3 mg/kg had 25% non-significant inhibition of AUC and the effect was similar to the groups treated with combination of MTX or MTX alone. A very similar trend was observed with final paw weights. Significant effect was observed with higher doses but not 3 mg/kg **11p** alone. Animals treated with **11p** alone had slight but non-significant improvement of body weight gain at 10 and 30 mg/kg.

The effects on histopathology indicate **11p** has disease modifying anti rheumatic drug (DMARD) activity at all doses, alone or in combination with MTX. The lack of a dose–response for the DMARD effects of **11p** suggest even 3 mg/kg achieves a maximal effect. Compound **11p** at all doses also had stronger DMARD activity than Methotrexate at 0.05 mpk.

Compound **11p** also was tested at 30 and 300 mg/kg po in guinea pig EAE.[20] In this model of EAE animals were immunized and only animals with established disease (clinical score[21] >0) were randomized into treatment group at 16–18 days post immunization. Compound **11p** was dosed orally bid for 12 days as Natalizumab (Tysabri) was dosed every other day. As illustrated in Figure 3, Natalizumab and high dose **11p** were able to reverse paralytic symptoms of EAE in guinea pigs as measured by clinical score. Mean clinical scores in the Natalizumab treated group were reduced from 1.3 before treatment to an average score of 0.5 by treatment day 12. 300 mg/kg **11p** administration reduced the mean clinical score by half from 1.3 to 0.7 over the treatment period. In guinea pigs treated with either vehicle or low dose **11p**, clinical scores increased steadily over the treatment period. Vehicle treated animals experienced an increase in mean clinical score from 1.2 to 1.6, while 30 mg/kg **11p** treated animal's scores likewise increased from 1.2 to 1.9 over the same period.

As illustrated in Table 5, the activities of **11p** on several integrins were determined. Compound **11p** showed no activity on LFA1[22] or α5β1.[23] Compound **11p** was much weaker in assays involving binding of αvβ1 to osteopontin (OPN),[24] or α4β7 to MadCAM.[25]

In conclusion, we have identified a group of arylsulfonamide pyrimidines, which are potent inhibitors of VLA-4. These compounds retain the potency of the similar dipeptide inhibitors, but display much greater bioavailability, and achieve efficacy in animal models of asthma, rheumatoid arthritis and multiple sclerosis when dosed orally.

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13. $\alpha_4\beta_1$-Dependent adhesion: Jurkat TM adhesion to human plasma fibronectin: 96 wells plate (Costar 3590) is coated with fibronectin (FN) (Calbiochem cat # 341635) at 1.25 μg/well in PBS++ (PBS + 1 mM $CaCl_2$ + 1 mM $MgCl_2$) overnight at 40 °C. Then the plate is blocked with assay buffer (Hepes 20 mM, NaCl 140 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM and 0.3% BSA) for 30 min at rt and washed 3 times with assay buffer. Calcein labeled Jurkat TM cells (Molecular probes cat # C-1430, according to manufacturer procedure), at 105 cells/well (final volume: 100 μl /well) in either assay buffer + 10 μg/ml TS2/16 (activating anti-α_4 antibody, Elan) or 100% human or rat serum (Seracare), are pre-incubated in a 96 wells Flexi plate with 5 μg/ml anti-α_5 (Pharmingen cat # 555614) and with a dose response of compound, for 30 min on wet ice. 21/6 (anti-α_4, Elan) at 10 μg/ml is used as a positive control to inhibit the $\alpha_4\beta_1$ dependent binding. Cells are then transferred to the FN coated plate and binding is allowed for 35 min at 37 °C, 5% CO_2 (incubator). Plate is washed 3 times with assay buffer and read for fluorescence (Applied Biosystems, CytoFluor series 4000).
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15. *Systemic exposure was determined as follow:* Three fasted male Sprague–Dawley rats were adminstered test compound mixtures at a total dose of 10 mg/5 mL/kg orally. Individual test compounds typically varied from 1.1 to 2.0 mg/kg. Blood samples were collected at 4, 8 and 12 h post dose by intracardiac puncture. Separated acidified plasma was extracted with etheyl acetate, evaporated to dryness and reconstituted in the chromatographic mobile phase and subjected to LC/MS/MS analysis on a PE-Sciex API-365 triple quadurpole mass spectrometer. Quantitation was performed with PE-Sciex software (MacQuan v1.6).
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21. Clinical scoreing criteria for guinea pig EAE: (0) No abnormality; (1) Abnormal gait. Pig may 'wobble' or lean to one side as it walks. Partial paralysis of one leg may also be present; (2) Bi-lateral hind limb weakness. Animal is unable to support its hind limbs consistently. Inability to right itself when placed on its back and held in hand; (3) Complete hind limb paralysis. Animal is unable to move hind limbs, motility limited to fore limbs only; (4) Moribund, that is, the animal is unable to right itself for locomotion, or complete paralysis of all limbs.

22. $\alpha_L\beta_2$-Dependent adhesion: 8866 cell adhesion to ICAM1-FC, protocol similar as described in Ref. 13.
23. $\alpha_5\beta_1$-Dependent adhesion: THP-1 cell adhesion to human plasma fibronectin, protocol similar as described in Ref. 13.
24. $\alpha_V\beta_1$-Dependent adhesion: 293/$\alpha_V\beta_1$ Transfected Cells Adhesion To Mouse-OPN-HIS, protocol similar as described in Ref. 13.
25. $\alpha_4\beta_7$-DEPENDENT ADHESION: 8866 cell adhesion to MadCAM-FC, protocol similar as described in Ref. 13.