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# Synthesis, characterization and evaluation of pro-drugs of VLA-4 antagonists

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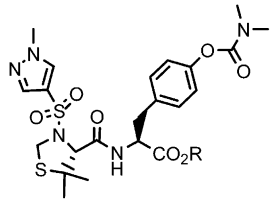
**Abstract**—A pro-drug strategy to identify orally efficacious VLA-4 antagonists is described. Potential pro-drugs were evaluated for their physical chemical characteristics and in vitro properties, including solubility, stability, permeability and plasma stability. Based on this characterization, promising compounds were identified for in vivo pharmacokinetic evaluation. These studies resulted in the identification of a pro-drug that exhibited desirable blood levels in PK studies in several different species.


The disruption of cell adhesion processes through pharmacological intervention holds significant promise for the treatment of inflammatory diseases. Of particular interest is the integrin, VLA-4, which was first shown to mediate cell trafficking, and more recently, has been shown to regulate the activation of a number of cell types.[1] Several reports describe the utility of VLA-4 antagonists (mAb's, peptides, and small molecules) which have shown efficacy in disease models of multiple sclerosis,[2,3] asthma,[4] and rheumatoid arthritis,[5] among others.[6] Unfortunately, many of the small molecules described, while exquisitely potent, suffer from low oral bioavailability and poor PK properties.[7–9] This paper describes our efforts to apply a pro-drug strategy to improve the PK characteristics and 'developability' of a potent VLA-4 antagonist.

A series of derivatives of proline-phenylalanine which potently disrupt VLA-4 mediated adhesion has recently been described by us[10] and others.[11] Of these derivatives, our attention was focused on compound **1**. This molecule inhibited VLA-4 mediated adhesion to HVEC's with an $IC_{50} = 6nM$. [12]



1: R= H
2: R= Et
3: R= iPr
4: R= $CH_2C(CH_3)_3$
5: R= $CH_2OCOC(CH_3)_3$
6: R= $CH_2CH_2OCH_2CH_2OCH_3$
7: R= $CH_2CH_2OPh$

**Figure 1.** Parent VLA-4 antagonist 1 and ester pro-drugs 2–7.

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**Table 1.** Physico-chemical properties of VLA-4 antagonist **1** and its pro-drugs

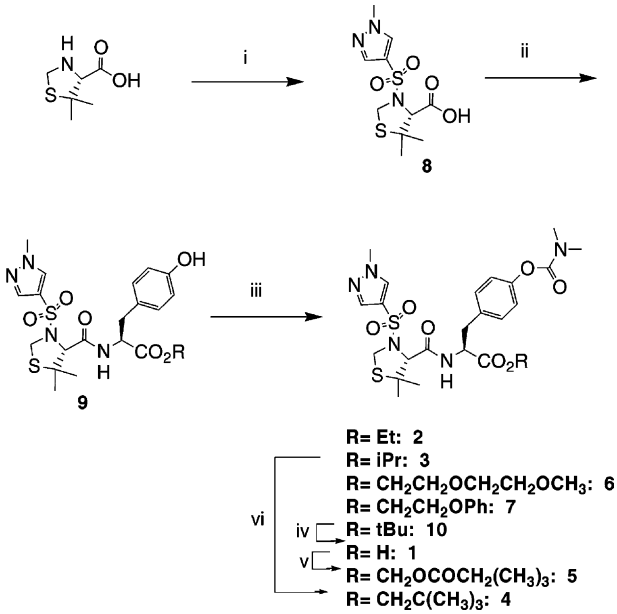
| Compd | SIF solubility[a] (mg/mL) | SGF solubility[a] (mg/mL) | SIBLM solubility[b] (mg/mL) | Caco-2[c] ($\times10^{-6}$ cm/s) | Log $D_{oct}$[d] (pH 4.5–6.5) | Rat plasma stability[e] ($t_{1/2}$ min) |
|---|---|---|---|---|---|---|
| **1** | >20 | 3.3 | >20 | 0.07 | | |
| **2** | 0.341 | 0.629 | 1.18 | 4.24 | 2.27–2.00 | 8.08 |
| **3** | 0.0455 | 0.0949 | 0.158 | 6.83 | 2.45–2.47 | 10.9 |
| **4** | Not detectable | 0.000334 | 0.00697 | 4.52 | 3.43–3.42 | 53.4 |
| **5** | Not detectable | 0.0130 | 0.119 | 2.49 | 3.10–3.17 | 2.82 |
| **6** | 2.4 | 2.83 | 4.65 | 2.03 | 1.41–1.42 | 13.1 |
| **7** | 0.000506 | Not detectable | 0.0240 | 7.58 | 3.21–3.34 | 1.6 |

[a] According to USP 23, pg. 2051.
[b] TenHoor, C. N.; Bakatselou, V.; Dressman, J. *J. Pharm. Res.* **1991**, 8, 1203.
[c] Ref 16; Metoprolol, used as a reference of a highly permeable compound (fa = 90–100), exhibited Mean Papp = 2×10–6 cm/s, which corresponds to 90–100% absorption; Mannitol, used as a paracellular reference marker with fa = 15, exhibited a Mean Papp = $0.3\times10^{-6}$ cm/s.
[d] $LogD_{octanol}$ was determined by HPLC peak area analysis of aqueous and octanol phases after 24 h shaking.
[e] Stock solutions of compound was added to rat plasma, and incubated at 37× for 24 h. Aliquots were removed at timepoints 0, 10, 20, 40, and 80 min and 3, 6, and 24 h. After extraction, centrifugation, evaporation and reconstitution with mobile phase, half-lives were determined by HPLC peak area analysis of ester and acid **1**.

To evaluate its potential to be developed as an oral agent, the physico-chemical properties of **1** were measured. Two p$K_a$'s[13] were measured at 11.0 and 2.9. The neutral species, which exists at low and high pH values, exhibits logP values in the range of 3–4. [14] These results indicate **1** will be amphoteric over the critical pH range 5–8, and predicts it will be poorly absorbed.[15] The amorphous compound exhibited good to high solubility in a variety of buffer systems (>20 mg/mL at pH 4.5–9), and in simulated physiological fluids (Table 1). Permeability in Caco-2 assays[16] was measured (Mean Papp = $0.07\times10^{-6}$ cm/s) and predicted poor permeability and <50% fraction absorbed in humans. The permeability rate was less than that observed for the paracellular reference marker Mannitol (Mean Papp = $0.3\times10^{-6}$ cm/s; 15% fraction absorbed in man). According to BCS System, the acceptable solubility but poor permeability of compound **1**, would result in a class III classification.

In order to enhance the permeability of this important compound, a pro-drug strategy was initiated. While a number of linkages were considered, preliminary data suggested esters as most promising. A series of esters (Fig. 1) were prepared including alkyl (ethyl **2**, isopropyl **3**, pivaloyl **4**), and substituted alkyl esters (2,2-dimethylpropionyloxymethyl **5**, methoxyethyoxyl ethyl **6,** 2-phenoxyethyl **7**). The synthesis of these derivatives and the parent acid is described in Scheme 1. 1-Methylpyrazole-4-sulfonyl chloride and L-(5,5-dimethyl)thiaproline were treated with $K_2CO_3$ in water to provide the sulfonamide **8**.[17] Coupling of **8** with the appropriate tyrosine ester (R = ethyl, isopropyl, ethoxyethoxylmethyl and phenoxyethyl, *t*-butyl) afforded the dipeptides of general structure **9**.[18] Treatment of **9** with dimethyl carbamoyl chloride and $K_2CO_3$ generated esters **2**, **3**, **6**, **7** or **10**. To prepare the acid **1**, **10** was treated with trifluroacetic acid. The acid was used as starting material to generate ester **5** via treatment of chlroromethyl pivalate and $K_2CO_3$.[19] Finally, transesterification of isopropyl ester **3** with pivalic alcohol and titaniumisopropoxide afforded ester **4**.

To evaluate their suitability, the esters were evaluated for their physical properties—particularly solubility in simulated physiological fluids, Caco-2 permeability, log D and plasma stability. Table 1 lists the data generated for these pro-drug esters. In all cases, Caco-2 data[16] indicated that the pro-drug strategy significantly improved permeability. Mean Papp values were all $>2\times10^{-6}$ cm/s indicating the potential for high permeability (>90% absorption). When measuring log $D_{octanol}$'s at pH's between 4.5 and 6.5, all compounds had values (ranging from 1.4–3.4) that fell within, or near the critical range of >0 and <3 that has been correlated with good oral absorption.[15] Solubility of the esters in a variety of simulated physiological fluids (simulated intestinal fluid, simulated gastric fluid, simulated bile lipid mixture) was measured. In comparison to the acid **1**, solubility diminished in all cases. In the case of the pivaloyl **4**, dimethylpropionyloxymethyl **5,** and 2-phenoxyethyl **7** esters, solubility was extremely poor, or undetectable.




**Scheme 1.** Synthesis of acid **1** and ester pro-drugs 2–7. (i) 1-methylpyrazole-4-sulfonyl chloride, potassium carbonate; (ii) L-tyrosine ester hydrochloride, *N*-methyl morpholine, 1-hydroxybenzotriazole, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; (iii) dimethyl carbamyl chloride, potassium carbonate; (iv) trifluoracetic acid; (v) potassium iodide, chloromethylpivalate, potassium carbonate; (vi) neopentyl alcohol, titanium(IV) isopropoxide.

To determine that the esters could indeed be cleaved in plasma to generate active parent, rat plasma stability studies were carried out. As expected, simple esters were rapidly cleaved with half lives in the 2–10 min range. Pivaloyl ester **4** exhibited significant stability ($t_{1/2} = 53.4$ min). The prolonged stability of this compound in rat plasma was predicted to correlate to lower levels of active acid in plasma, and even lower levels in higher species, and therefore the compound was removed from further consideration.

Compounds **5** and **7** were de-prioritized due to lack of solubility under conditions that mimic the GI tract. Based on a suggested minimum thermodynamic solubility of 50 μg/mL[15b] esters **2**, **3**, and **6** (aqueous solubility = 0.473 mg/mL, 0.069 mg/mL and 5.38 mg/mL, respectively) were predicted to be the most promising for oral delivery, however, **6** existed as an amorphous solid, and it too was dropped from further consideration.

Due to their acceptable solubility, good Caco-2 permeability, short half-life in rat plasma, and optimal logD, the ethyl (**2**) and isopropyl (**3**) esters were chosen for further evaluation. Both compounds were dosed orally at 30 mg/kg to male rats and mean blood concentrations of parent and active acid were measured.[20] In the case of **2**, significant concentrations of acid parent were detected at time points of 5 min though 24 h, with a peak level of 0.31 μg/mL at 15 min. No ester was detected. When the *iso*-propyl ester **3** was dosed, both ester and acid were detected at all times points (5 min to 24 h). The ester was quantitated between time points of 5 min and 8 h, albeit at consistently low levels (0.001–0.004 μg/mL). The acid was quantitated at acceptable levels (0.014–0.382 μg/mL) from 5 min (0.2 μg/mL) through 4 h (0.014 μg/mL), with a peak level of 0.38 μg/mL at 15 min. Similar results in another animal species (results not shown) indicate that the pro-drug optimization was not species specific. These concentrations of active parent are significantly higher than the $IC_{50}$ for VLA-4 inhibition, and were therefore predicted to result in vivo efficacy in disease models mediated by VLA-4. The translation of these blood levels into oral efficacy in animal models of disease of *iso*-propyl and ethyl ester pro-drugs will be reported elsewhere.

In summary, a pro-drug strategy was employed to impart desirable physico-chemical and PK properties to a potent VLA-4 antagonist. By evaluation of their physical properties, pro-drugs with the most promising properties (solubility, permeability, logD, plasma stability) could be prioritized for further testing. These predictions were confirmed in in vivo PK studies. The ability to rapidly evaluate a series of pro-drugs using in vitro assays can be used to identify the most promising compounds on which to perform more elaborate in vivo assays.

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