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The Discovery of a Potent, Intracellular, Orally Bioavailable, Long Duration Inhibitor of Human Neutrophil Elastase—GW311616A a Development Candidate

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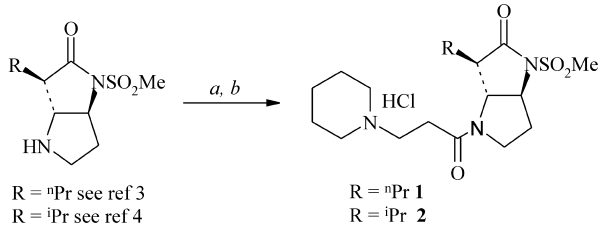
Abstract—The discovery of a potent intracellular inhibitor of human neutrophil elastase which is orally active and has a long duration of action is described. The pharmacodynamic and pharmacokinetic properties of a *trans*-lactam development candidate, GW311616A, are described.

A considerable body of work now describes inhibitors of human neutrophil elastase (HNE),[1] a serine protease from the trypsin class, for the treatment of respiratory diseases such as chronic bronchitis, cystic fibrosis and emphysema. We introduced *trans*-lactams as inhibitors of HNE and described some of their properties.[2] Whilst some of these compounds (e.g., **1**) are orally active with reasonable bioavailability in hamster, the in vivo oral doses required to significantly inhibit elastase are high (20 mg/kg) and the compounds are cleared at rates greater than liver blood flow. We describe here analogues of these compounds that are more potent and that have lower clearances. The properties of a development candidate, GW311616A, are also described.

Improving Blood Stability and Reducing Clearance

We have described[2] elsewhere that a major component to the high clearance of *trans*-lactams after oral dosing in hamster is lactam ring-opening. To minimise clearance, we considered structural modifications to either side of the lactam carbonyl group. As we had optimised previously the activating group on the lactam *N*,[2] we elected to modify the substituent α to the lactam carbonyl group. We reasoned that replacement of the *n*-propyl group[3] with an isopropyl group[4] (as in **1** to **2**, Scheme 1) would offer greater steric protection of the carbonyl to the ring-opening hydrolysis process[5] whilst maintaining inhibitory activity. Endogenous substrates for HNE include Val as a P1 residue. This minor structural change was a major synthetic challenge,[4] but had the desired effect in promoting a substantial increase in hydrolytic stability in the presence of blood and plasma (Table 1).



Scheme 1. (Structures racemic) (a) 3-piperidinopropionic acid, $Me_2N(CH_2)_3NCNEt{\cdot}HCl$ (EDC), CH_2Cl_2; (b) HCl, Et_2O, CH_2Cl_2.

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Table 1. Pharmacokinetic properties of **1** and **2**

	R	HNE IC_{50}	Half-life in blood (h)				% inhib. hamster BAL po[b]		Clp[c]	F[c]
		nM[a]	Rat	Hamster	Dog	Human	2.2 mg/kg	20 mg/kg	mL/min/kg	%
1	^{n}Pr	120	0.3	1.4	3.5	3.7	4	75	140	49
2	^{i}Pr	242	3.5	>6	>6	>6	20	88	60	70

[a]Values are a mean of three experiments after 15 min preincubation. See ref 3 for experimental details.
[b]Inhibition of intraneutrophil elastase in hamster bronchoalveolar lavage (BAL), 6 h after both oral dosing of compound and intratracheal dosing of IL-8 (see ref 2 for details).
[c]Pharmacokinetic parameters were measured in hamster after doses of **1** at 3 mg/kg iv and 10 mg/kg po and **2** at 3 mg/kg iv and 3 mg/kg po.

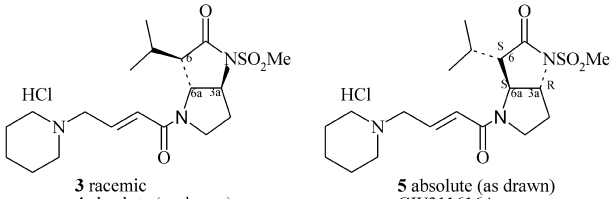
Table 2. In vitro and in vivo differences between the racemate **3** and enantiomers **4** and **5**

	Stereochemistry	HNE IC_{50} (nM)[a]	HWB IC_{50} (μM)[b]	% inhib. hamster BAL po[c]	
				0.73 mg/kg	2.2 mg/kg
3	Racemic	26	1.8	33	77
4	6*R*,6a*R*,3a*S*	274	1.4	17	34
5	6*S*,6a*S*,3a*R*	22	0.67	47	92

[a]Values are a mean of three experiments after 15 min preincubation. See ref 3 for experimental details.
[b]The compound is incubated with human whole blood (HWB) for 30 min. The white cells are then separated, washed, lysed and any residual elastase activity then measured (see ref 2 for details).
[c]Inhibition of intraneutrophil elastase in hamster bronchoalveolar lavage (BAL), 6 h after both oral dosing of compound and intratracheal dosing of IL-8 (see ref 2 for details).

Improving In Vivo Potency

The in vivo potency was the major remaining shortcoming. By this stage we had extensively explored, through a large structural array, both the nature of the base and length of the side chain (pK_a and log P/D) and found only marginal differences in in vivo potency. However, we discovered serendipitously that conversion of the 3-piperidinopropionamide **2** into the corresponding piperidinocrotonamide **3** resulted in a log unit increase in activity. SARs indicated that the pharmacodynamics required the presence of unsaturation in the side chain to provide either necessary π character and/or conformational rigidity. With the impressive in vivo profile of **3**, we prepared its enantiomers **4** and **5**.[4] The 6*S*,6a*S*,3a*R* isomer is significantly the more active enantiomer (Table 2).



3 racemic
4 absolute (as drawn)

5 absolute (as drawn)
GW311616A

Properties of 5-GW311616A

Having achieved the target in vivo potency, we explored more fully the properties of **5** to determine whether it met our required development candidate criteria.

Co-crystallisation with Porcine Pancreatic Elastase (PPE)

A 1.7 Å resolution X-ray structure of GW311616A co-crystallised with PPE[6] at pH 4.6 reveals the mode of binding of the inhibitor and provides a firm basis for analysis of analogous protein–ligand interactions in human neutrophil elastase. High-quality electron density covers the whole inhibitor molecule and reveals its interactions with the active site (Fig. 1), including a covalent bond with Ser203 (PPE numbering) and the hydrogen bond with the main-chain nitrogen atom of Val224. The ester carbonyl oxygen is pointing towards the oxyanion hole (Gly201 and Ser203), but the distance is too long for good hydrogen bonding with the corresponding nitrogen atoms. There is also an indirect hydrogen bond between the nitrogen atom of the piperidine ring and the carbonyl oxygen of Val224, bridged by a water molecule. There is good shape complementarity and a number of favourable hydrophobic

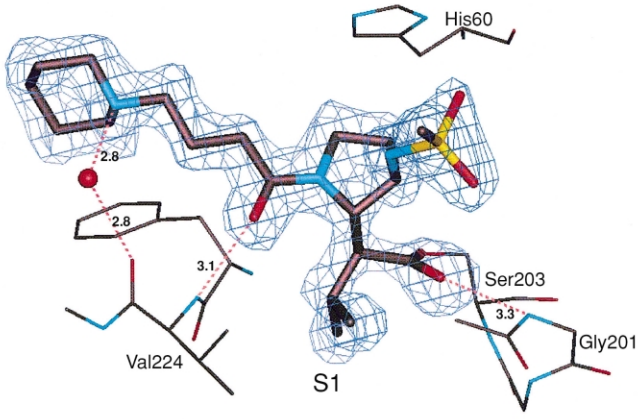


Fig. 1. Crystal structure of GW311616A complexed with porcine pancreatic elastase (PPE). The bold structure is the ring opened analogue of **5** covalently bound to Ser203. Key residues of PPE are drawn with thin bonds and the hatch shading represents areas of electron density. S1 is the primary enzyme specificity pocket. The dotted red lines represent H-bonding distances measured in angstroms and the red dot is a water molecule.

interactions around the piperidinyl ring of the inhibitor in the S4 pocket involving Phe223, Val103 and Trp179.

The catalytic triad histidine (His60) is displaced from its catalytic position, contributing to the stability of the complex. Although the isopropyl group does not fill the S1 pocket completely, it is involved in a number of favourable hydrophobic interactions, especially with Val224. Comparison of the PPE-GW311616A structure with the crystal structure of HNE (Protein Data Bank entry 1HNE) indicates that the protein–inhibitor interactions observed in PPE would be very similar in HNE. Similarity between the active sites of HNE and PPE was pointed out by Navia et al.[7]

In Vitro Potency, Selectivity and Kinetics

Compound **5** is a potent intracellular inhibitor of HNE (see Table 2). The HNE inhibitor **5** is selective over other human serine proteases (IC_{50} values >100 μM for trypsin, cathepsin G, and plasmin, >3 μM for chymotrypsin and tissue plasminogen activator). Acetylcholinesterase is not inhibited by **5** at 100 μM.

HNE enzyme kinetic data (Table 3) suggest that **5** is more potent than the trifluoromethylketone inhibitor ZD8321 ($K_i = 13$ nM).[8] Although the association rates of **5** are slower than those published for the β-lactam-based L-694,458 (under the authors' conditions, $k_{obs}/[I] = 3.78 \times 10^6\ M^{-1}\ s^{-1}$),[9] the potencies of the two compounds in the HWB assay are very similar (IC_{50} values 0.67 and 0.45 μM, respectively).

Both competitor HNE inhibitors (L-694,458 and DMP777) have been reported as being evaluated in clinical trials.

Pharmacodynamic Parameters

The dose–response and duration of action of orally dosed **5** were determined in dog by measurement of intraneutrophil elastase activity in blood samples (Fig. 2—see ref 2 for details). Myeloperoxidase (MPO) activity was measured in the same samples to correct for any variation in recovery of neutrophils. At 0.22 mg/kg, greater than 50% inhibition of elastase is achieved 6 h after dosing, with activity returning towards control values relatively quickly. By contrast, a single oral dose of 2 mg/kg rapidly abolishes circulating enzyme activity, and greater than 90% inhibition is maintained for 4 days. This prolonged effect is believed to be due to penetration of neutrophils in the bone marrow by **5**, with subsequent release of cells containing pre-inhibited elastase into the circulation. Experiments with **5** in the hamster have confirmed that elastase activity is inhibited in the bone marrow.[11] Comparison of the inhibitory and pharmacokinetic profiles of **5** demonstrates that its duration of action is independent of its half-life (1 h in dogs) which is consistent with the essentially irreversible nature of the inhibition.

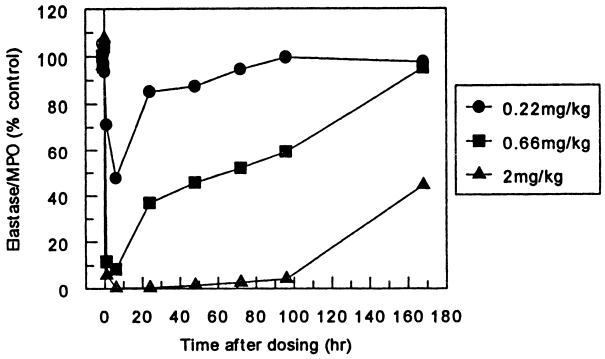


Fig. 2. Effect of orally dosed GW311616A on intracellular elastase in the dog.

Table 3. Kinetic parameters of **5** measured at pH 7.4

$k_{obs}/[I]$	k_{on} ($s^{-1}\ M^{-1}$)	k_{off} ($\times 10^{-6}\ s^{-1}$)	K_i (nM)[a]	$t_{1/2}$[b] (h)
753	6630	2.07	0.31	93.0

[a] $K_i = k_{off}/k_{on}$, k_{off} and k_{on} are, respectively, the dissociation and association rate constants.[10]
[b] $t_{1/2} = 0.693/k_{off}$ represents the half-life time of the acyl–enzyme complex.

Table 4. Pharmacokinetic parameters of GW311616A in rat and dog

Species	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	Clp (mL/min/kg)	Vd_{area} (L/kg)	$T_{1/2}$ (h)	F %
Rat	2	232	0.5	79	7.5	1.1	60
Dog	2	461	0.7	28	3.5	1.5	~100

Pharmacokinetic Parameters

5 is orally bioavailable in rat, dog (Table 4) and hamster (data not shown) despite moderate to high plasma clearance, which indicates that clearance is predominantly extrahepatic.

Effect of 5 on Infection

Since neutrophil elastase is involved in host-defense mechanisms, we studied the effect of **5** on infection. Preincubation of neutrophils with **5** did not affect their ability to kill a wide range of organisms (*Candida albicans, Escherichia coli, Haemophilus influenzae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae*). Similarly, predosing of hamsters with **5** did not affect clearance of intraperitoneal infections of *Escherichia coli* or *Staphylococcus aureus*.

Physicochemical Properties of 5

5 shows no significant degradation at room temperature for over 12 months. It has aqueous solubility in excess of 20 mg/mL and is non-hygroscopic. Its m log D is 0.46 and it has a measured pK_a of 8.44. In numerous batches prepared to date, **5** exists in a single crystal form.

Conclusion

The properties of compound **5** (GW311616A) described here fulfilled our criteria for a development candidate.

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