Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 243–246

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Intracellular Inhibition of Human Neutrophil Elastase by Orally Active Pyrrolidine-*trans*-lactams

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Received 26 September 2000; accepted 8 November 2000

Abstract—Described are the acylation binding of *trans*-lactam **1** to porcine pancreatic elastase, the selection of the SO_2Me activating group for the lactam *N* which also confers metabolic stability in hamster liver microsomes, the introduction of aqueous solubility through the piperidine salt **9**, the in vivo oral activity of **9** and its bioavailability, and the introduction of **9** as an intracellular neutrophil elastase inhibitor.

Introduction

There is now a considerable body of work describing inhibitors of human neutrophil elastase (HNE),[1] a serine protease belonging to the trypsin class, for the treatment of respiratory diseases such as chronic bronchitis, cystic fibrosis and emphysema. In a previous paper[2] we introduced pyrrolidine *trans*-lactones and lactams as new pharmacophores for elastase inhibition. Despite the plethora of published HNE inhibitors,[1] there is very little work[3] describing *intracellular* inhibitors, where the elastase is inhibited whilst still packaged within the unactivated neutrophil. Further, only a limited number of published elastase inhibitors have oral activity.[1] We describe in this paper the medicinal chemistry process followed in discovering *trans*-lactams which are both intracellular and orally active neutrophil elastase inhibitors. The intracellular mechanism has far-reaching pharmacokinetic and pharmacodynamic implications that we have exploited as described in this paper. The starting compound was the previously described lactam **1**.[2]

We outline here the crystal structure of **1** with porcine pancreatic elastase (PPE), optimisation of the lactam *N* activating group, introduction of water solubility, improvement to the metabolic stability and details of intracellular inhibition.

Crystal Structure of 1 with PPE

Figure 1 shows a detail of the crystal structure of PPE complexed with **1** at pH 7.6[4] that provided valuable structural information. Observed binding features of **1** include: (a) a covalent bond between Ser195 and the carbonyl of the ring opened *trans*-lactam; (b) the allyl group in the S1 pocket; (c) hydrogen bonds between the ester carbonyl of the inhibitor and the main chain nitrogen atoms of Gly193 and Ser195 (the oxyanion hole); (d) the rotation of His57 out of its catalytic position and its replacement by a water molecule; (e) an absence of electron density for the benzyloxycarbonyl group; and (f) poor electron density for the methylsulphonamide group. Mass spectra analyses of the complex confirmed Ser195 acylation. The His57 displacement contributes to the complex stability. This information was invaluable in

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PII: S0960-894X(00)00632-6

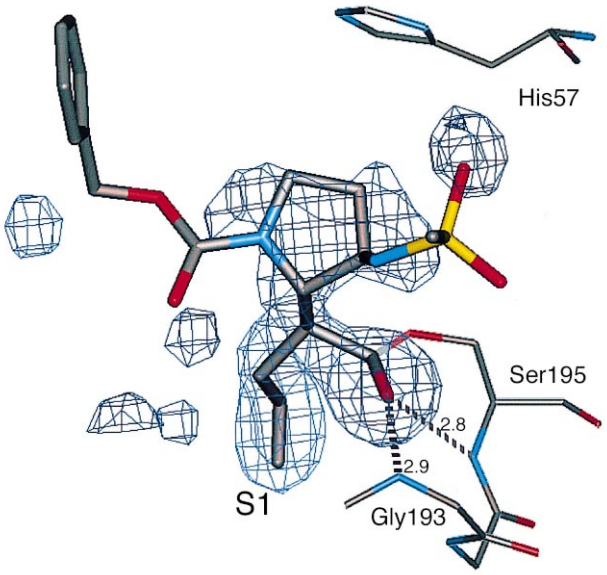


Figure 1. Details of the crystal structure of **1** with PPE. The bold structure is the ring opened analogue of **1** covalently bound to Ser195. Key residues of PPE are drawn with thin bonds and the hatch shading represents areas of electron density. S1 is the primary enzyme specificity pocket. The distances between the lactam carbonyl oxygen and the NHs of Gly193 and Ser195 are measured in angstroms. The electron density near one of the sulphonyl oxygens is the water molecule displacing His57.

determining a medicinal chemistry strategy for drug discovery where each substituent of the *trans*-lactam template could be optimised iteratively. In particular, variation of the pyrrolidine substituent could be used to improve specific pharmacokinetic and dynamic properties due to its apparent lack of participation in enzyme binding.

Optimisation of the Activating Group

We first explored the lactam *N* substituent (the activating group), which was introduced typically by deprotonation of **2** followed by addition of an electrophile (Scheme 1). The SAR (Table 1) from these compounds revealed two requirements. Firstly an activating electron withdrawing group is essential for activity and secondly the substituent atom proximal to the lactam *N* should have tetrahedral geometry.[5] In general, as a class, these compounds are selective HNE inhibitors over other serine proteases (data not shown).

Table 1. In vitro HNE activities

	R	HNE IC_{50} (μM)[a]
1	SO_2Me	0.047
2	H	>500
3	$^nC_6H_{13}$	29
4	CO_2Et	2
5	CONHEt	31
6	COMe	0.69
7	SO_2-2-Naphthyl	0.036

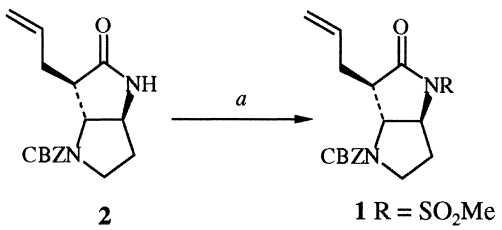
[a]IC_{50} values were used for ranking purposes only. Values are a mean of three experiments. 50 mM Tris/HCl (pH 8.6), 150 mM NaCl, 11.8 nM purified HNE and water dilutions of inhibitor from a 10 mM stock solution in DMSO are incubated for 15 min at 30 °C. After the addition of 0.6 mM MeO-Succ-Ala-Ala-Pro-Val-*p*-nitroanilide the residual elastase activity is measured. See ref 2 for experimental details.

Introducing Water Solubility and Oral Activity

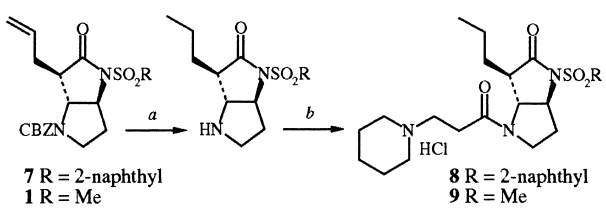
On potency grounds, **1** and **7** were selected as leads reasoning that modification of the naphthalene ring of **7** offered flexibility for adjusting molecular properties that might later be required. Conscious that aqueous solubility of a compound greatly facilitates its biological testing and development, we decided to introduce a tertiary amine into the molecule as its salt to enhance solubility. Deprotection of the benzyl carbamates **1** and **7** (with concomitant reduction of the allyl group), coupling 3-piperidinopropionic acid using EDC and treatment of dichloromethane solutions of the free bases with ethereal hydrogen chloride gave the hydrochlorides **8** and **9** (HNE IC_{50} 137 nM and 140 nM, respectively) (Scheme 2). Both compounds have >1 mg/mL solubility in water. Compound **8**, when tested in vivo,[6] is orally active as determined by inhibition of elastase in hamster bronchoalveolar lavage (BAL) 6 h after an intratracheal dose of IL-8 (60% inhibition at 40 mg/kg, 30% at 20 mg/kg and 17% at 10 mg/kg).

Improving Metabolic Stability

In seeking to understand the weak oral activity of the piperidine derivative **8**, it was found that it is metabolically unstable in hamster liver microsomes (78% turnover at 50 μM, 20 min).[7] In contrast, the methyl sulphonamide analogue **9** proves to be stable in hamster liver microsomes (0% turnover) and more potent orally (76% and 41% elastase inhibition at 20 and 10 mg/kg, respectively). The methanesulphonyl group thus became the preferred lactam activating group for the rest of the medicinal chemistry programme.



Scheme 1. (Compounds racemic). Reagents: [a] LHMDS, THF, −78 to 0 °C then recool to −78 °C, add electrophile, warm to rt; or NaH, THF or DMF, 5 °C, add electrophile, warm to rt.



Scheme 2. (Compounds racemic). [a] $Pd(OH)_2$, H_2, THF; [b] 3-piperidinopropionic acid, $Me_2N(CH_2)_3NCNEt{\cdot}HCl$ (EDC), CH_2Cl_2; [c] HCl, Et_2O, CH_2Cl_2.

Pharmacokinetic Parameters of 8 and 9

Encouraged by the improvement in oral activity, the pharmacokinetic parameters of **8** and **9** were measured (Table 2). The volume of distribution[8] of **9** is smaller than **8** and although the clearance of **9** still exceeds liver blood flow, it is better than **8**. Given this high clearance, the 49% bioavailability of **9** is surprising and suggests that clearance is not predominantly hepatic, in keeping with the stability evident in vitro in hamster liver microsomes. The principle route of metabolism of **8**, which contributes to the very high clearance, is ring opening of the activated lactam.[9] Our success in overcoming this issue will be described elsewhere. An intriguing feature of the in vivo studies was the discovery that despite the short half lives of **8** and **9**, their inhibitory effect on elastase in circulating neutrophils is prolonged (>20 h). We have attributed this feature to the mode of action of these compounds as irreversible intracellular elastase inhibitors.

Intracellular Inhibitors

Intracellular elastase inhibitors described herein have the ability to penetrate rapidly the circulating neutrophil, and enter the azurophilic granules where they inhibit the stored elastase, such that when the neutrophils are recruited and activated, inhibited elastase is released (Fig. 2).[10] Drug distribution into (and out of) cells is usually an equilibrium process — in this case the absorption process is irreversible due to covalent binding of the inhibitor to intracellular elastase. This mode of inhibition pays a rich dividend in lowering the pharmacokinetic requirements, providing the rate of neutrophil penetration and inhibition is competitive with respect to clearance mechanisms. There is no requirement for sustained circulating therapeutic concentrations of the compound because the inhibitor has already performed its function which persists for the lifetime of the neutrophil.

Table 2. Pharmacokinetic parameters of **8** and **9** determined in hamster

Parameter	**8** iv (4 mg/kg)	**8** po (40 mg/kg)	**9** iv (3 mg/kg)	**9** po (10 mg/kg)
Clp (mg/min/kg)	200		140	
Vd (L/kg)	8.1		2.3	
$t_{1/2}$ (h)	0.5		0.2	0.5
F (%)		1		49

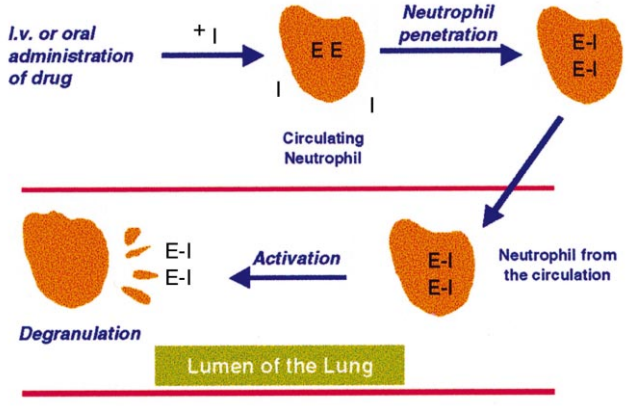


Figure 2. A schematic representation of intracellular inhibition. I represents the inhibitor and E the elastase. The orange blobs represent neutrophils. The inhibitor penetrates the circulating neutrophil, inhibits the elastase such that on recruitment into the lung and degranulation of the neutrophil, only inhibited elastase is released.

Table 3. IC_{50} values in human whole blood (HWB),[11] a measure of intracellular activity (values are a mean of three experiments)

Compound	**1**	**7**	**8**	**9**	**10**[c]	**11**[d]
HWB IC_{50} (μM)	98%[a]	92%B, 23%P[b]	1.0	3.0	0.45	>10

[a]Percentage inhibition in whole blood at 10 μM.
[b]As for [a] except in buffer (B) and plasma (P).
[c]Merck L-694,458.[12]
[d]Zeneca ZD-8321.[13]

An in vitro assay for intracellular inhibition has been developed,[11] where the inhibitor compound is incubated with human whole blood for 30 min. The neutrophils are then separated, washed, lysed and any residual elastase activity then measured. We also evaluated the Merck (L-694,458)[12] and Zeneca (ZD-8321)[13] elastase inhibitors; only the former is a potent intracellular inhibitor (Table 3). Within a particular *trans*-lactam series (e.g., variation in the tertiary amine), it was observed that HWB activity correlated with mlogD. However plotting clogP versus HWB activity for all *trans*-lactams for which data was available (>100 compounds) showed no correlation.

Conclusion

This work laid the foundation for the final phases of our programme (further lowering clearance and increasing in vivo potency) that led to a candidate suitable for development — GW311616A that will be described in due course.

Acknowledgements

We would like to thank Terry Haley for the PPE mass spectrometry experiments.

References and Notes

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4. Racemic **1** was used during co-crystallisation with PPE; the *S*-allyl enantiomer was bound. As there is poor electron density for the benzyloxycarbonyl part of the inhibitor, details of an analogous structure (GW311616) of excellent quality will be published shortly.

5. The exception to this is the smallest acyl derivative, formyl, which is active (HNE IC_{50} 94 nM).

6. Adult hamsters were dosed orally with compound followed immediately by an intratracheal dose of human interleukin-8 (1 μg). After 6 h the lungs were lavaged with saline (2×2.5 mL). The neutrophils were separated, sonicated and centrifuged to yield the intracellular granules. These were disrupted by freeze-thawing and sonication. Residual elastase activity was then measured (see ref 2). For fuller details see PCT Int. Appl. (1999) WO 9912933 A2 19990318.

7. The compounds were incubated for 20 min in the S8 fraction of hamster liver microsomes and the disappearance of parent determined by HPLC/mass spectroscopy.

8. This may reflect lowering in logD of **9** (mlogD = −0.17) over **8** (mlogD = 2.70).

9. This was determined by HPLC and MS profiling of excreta after oral and intravenous administration of radiolabelled **8** to hamster.

10. Neutrophil function appears unaffected and the radioactivity of radiolabelled inhibitor can be seen concentrated in the granules of the neutrophil.

11. Heparinised whole blood (200 μL) was incubated with diluted samples of compound for 30 min at 37 °C. Red cells were then lysed with 750 μL of buffer (155 mM NH_4Cl, 0.12 mM EDTA, 10 mM K_2CO_3), at 4 °C for 15 min. The samples were then centrifuged, and the pellets washed three times with saline (300 μL). Resuspension of the final pellet in buffer (200 μL, 100 mM Tris, 300 mM NaCl, 1% (w/v) HTAB). The samples were then freeze-thawed four times and elastase activity determined by colourimetric assay as described in ref 2. For fuller details see PCT Int. Appl. (1999) WO 9912933 A2 19990318.

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