Design of Orally Active, Non-Peptidic Inhibitors of Human Leukocyte Elastase

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Human leukocyte elastase (HLE; EC 3.4.21.37) is a serine protease released by polymorphonuclear leukocytes in response to inflammatory stimuli.[1] Excessive elastolytic activity has been implicated in the etiology of a number of diseases such as adult respiratory distress syndrome,[2] cystic fibrosis,[3] and emphysema.[4] It has been postulated that a low molecular weight inhibitor of elastase could prove therapeutically useful against such pathologies.[5] ICI 200,880 (1),[6] a peptidic trifluoromethyl ketone (TFMK), is a potent inhibitor of HLE[7] that is currently being evaluated in clinical trials.

In animal models, aerosol administration of ICI 200,880 provides long-lasting protection against HLE-induced lung damage, but the compound has not shown useful levels of oral activity. We sought to design an orally bioavailable inhibitor of HLE by decreasing the peptidic character of ICI 200,880.

TFMKs serve as mechanism-based inhibitors of serine proteases by providing an electrophilic carbonyl for covalent attachment to the active site Ser-195. The formation of a stable, tetrahedral, hemiketal adduct has been demonstrated by crystallographic studies of one of our inhibitors (Ac-Ala-Pro-Val-TFMK, 2) complexed with the closely related enzyme, porcine pancreatic elastase (PPE).[8] Figure 1 illustrates key enzyme–inhibitor interactions that are observed in this crystal structure. Ser-195 is covalently attached to the TFMK carbonyl, and the resulting oxyanion is stabilized by hydrogen bonds to the backbone amide N-H of both Ser-195 and Gly-193. The isopropyl group of valine occupies the S_1 specificity pocket, and proline rests in the S_2 pocket of the enzyme. A pair of β-sheet hydrogen bonds are formed between the P_3 alanine and Val-216. The incorporation of these important recognition elements formed the focus for our design of new, non-peptidic TFMK inhibitors.

Modeling studies that docked inhibitor 2 into the active site of HLE (taken from an X-ray crystal structure[9]) indicated that the critical enzyme–inhibitor interactions could be maintained. Inspection of the conformation of the P_2 and P_3 residues of the bound inhibitor in this model suggested it would be possible to change the hybridization of the alanine α-carbon from sp^3 to sp^2 and build a hydrocarbon bridge from it to the adjacent proline

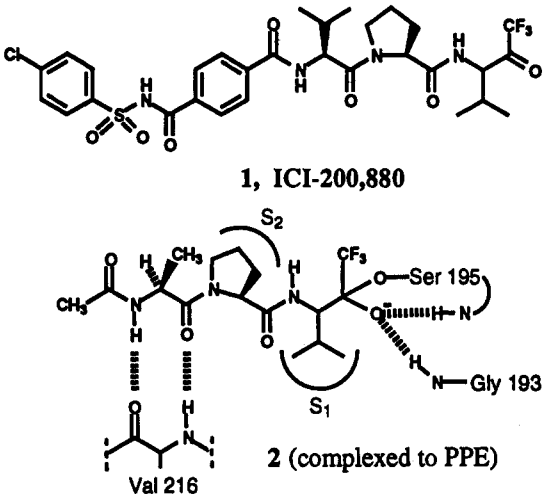

Figure 1. Peptidic TFMK inhibitors of HLE.

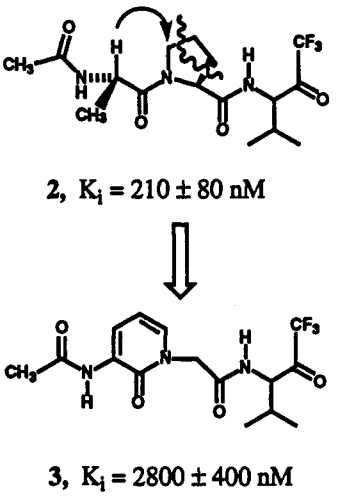

Figure 2. Design of non-peptidic inhibitors.

methylene, without disrupting the critical hydrogen-bonding interactions with Val-216 (Figure 2). Furthermore, it had been established that the proline in peptide inhibitors could be replaced by *N*-alkylglycine residues,[10] suggesting that cleavage and deletion of a portion of the proline ring would be feasible.

A substituted pyridone, 3-amino-2-oxo-1,2-dihydro-1-pyridylacetic acid, was proposed as an achiral, peptidomimetic for the Ala-Pro dipeptide portion of the TFMK inhibitor.[11] The validity of the design concept was corroborated by the demonstration that pyridone 3[12] (Figure 2) retained considerable activity against HLE. Modeling of a pyridone in the active site suggested that the absence of the proline ring might diminish the hydrophobic interaction with the S_2 subsite of the enzyme. In an attempt to regain that beneficial binding, hydrophobic substituents were introduced on the pyridone ring. The 6-phenylpyridone 4 (where the *N*-acetyl group has also been replaced with the *N*-CBZ moiety, which provides greater potency[13]) proved to be particularly active in vitro, exhibiting a K_i of 4 nM (Table 1). Although pyridone 4 did not inhibit a number of other proteases, it was relatively effective (K_i = 55 nM) against bovine pancreatic chymotrypsin (BPC). While inhibition of a *bovine* enzyme was not in itself a worry, it did raise some concern that the selectivity profile opposite *human* proteases might not be as robust as desired. Incorporation of a *p*-chloro sub-

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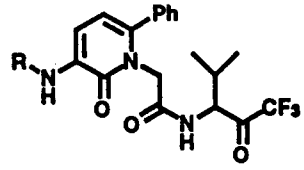
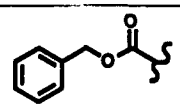
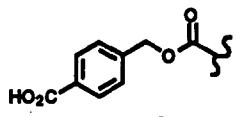
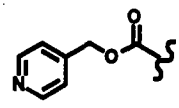
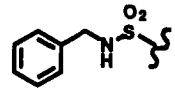
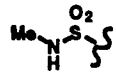
Table 1. N-Substituents on 6-Phenylpyridones

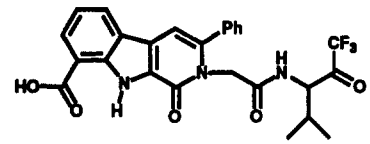


no.	R	formula[a]	K_i (nM)[b]	oral activity[c] % inhib/ dose (mg/kg)
4		$C_{27}H_{26}F_3N_3O_5$	4.5 ± 0.8	NS/20
5		$C_{28}H_{26}F_3N_3O_7$	5.7 ± 1.5	NS/20
6		$C_{26}H_{25}F_3N_4O_5$	8 ± 1	59/20
7		$C_{28}H_{27}F_3N_4O_5S \cdot 0.25H_2O$	1.8 ± 0.7	43/5
8		$C_{20}H_{23}F_3N_4O_5S$	18 ± 3	43/5

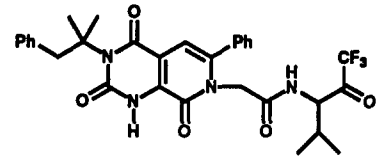
[a] Elemental analyses (C, H, N) were in agreement with the indicated formulas. Formulas for those compounds not listed in Tables 1 and 2 are given in ref 12. [b] The inhibition constants (K_i) versus HLE were determined by the method described in ref 7. Each number is the average of three determinations. [c] Oral activity was measured in an acute hemorrhagic assay as described in ref 15. The percent inhibition ($p < 0.05$, standard Student's t test) of HLE-induced lung hemorrhage provided by the indicated oral doses (mg/kg) is given. NS designates no significant activity.

stituent on the phenyl ring increased selectivity for HLE vs BPC by a factor of ten.[14]

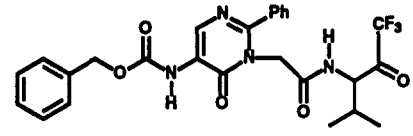
While the level of in vitro activity that had been achieved with pyridone **4** was exciting, the compound was not orally active at a dose of 20 mg/kg in an acute hemorrhagic assay (AHA).[15] The lack of activity was ascribed to a combination of relatively high lipophilicity (log $P > 4$ in octanol/H_2O) and low solubility (1.8 μg/mL in buffered saline). A number of chemical strategies were undertaken to address this deficiency. Aqueous molecular dynamics simulations[16] of HLE–inhibitor complexes indicated that the N-substituent (for example, the CBZ group of **4**) was conformationally mobile, spending considerable time in solution rather than anchored to the enzyme surface. Furthermore, the N-substituent was amenable to a variety of synthetic modifications, so it was targeted as a particularly appropriate region for introducing structural changes to modify the overall physical properties of the molecules. Diverse functionality (including acidic, neutral, and basic groups) did prove to be well tolerated on the N-substituent; inhibition constants for representative compounds are given in Table 1. More importantly, oral activity at doses of 5 mg/kg was observed for compounds such as **7** and **8**. A crystal structure of inhibitor **8** complexed to PPE provided further supportive evidence that these pyridones were able to bind to elastase in a manner similar to that observed for peptide **2** (Figure 1).



9, $K_i = 13 \pm 2$ nM



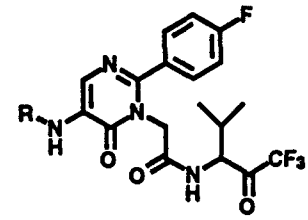
10, $K_i = 0.93 \pm 0.38$ nM

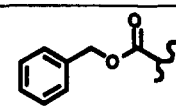
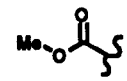


11, $K_i = 6.6 \pm 1.4$ nM

Figure 3. Heterocyclic alternatives to pyridones.

Table 2[a] N-Substituents on 6-(4-Fluorophenyl)pyrimidones



no.	R	formula	K_i (nM)	oral activity % inhib/ dose (mg/kg)
12		$C_{28}H_{24}F_4N_4O_5 \cdot 0.4H_2O$	8.2 ± 1.9	63/20 NS/5
13		$C_{20}H_{20}F_4N_4O_5$	40 ± 7	39/2.5
14	H	$C_{18}H_{18}F_4N_4O_3 \cdot 0.75H_2O$	101 ± 10	43/2.5 49/2.5[b]

[a] See footnotes for Table 1. [b] The interval between inhibitor dosing and HLE challenge was increased from 30 to 90 min in this case.

Concurrently, other heterocycles incorporating the pyridone design features (for example, carbolines **9**, quinazolinediones **10**, and pyrimidones **11**) were explored (Figure 3). Pyrimidones[17] provided attractive oral profiles; for example, the 6-phenyl-*N*-CBZ-pyrimidone **11** was active at 20 mg/kg. A number of alternatives to the 6-phenyl substituent on the pyrimidone were explored. A 4-fluorophenyl group, as in analog **12**, conveyed particularly good selectivity (BPC/HLE K_i ratio = 378), so that series was chosen for further structure–activity studies of the N-substituent in order to improve oral activity (Table 2). As expected from previous structure–activity studies, in vitro potency tended to diminish as the size and lipophilicity of the N-substituent decreased. However, smaller groups proved beneficial for conveying oral activity, as illustrated with two representative examples, **13** and **14**, in Table 2. Since it was desirable to maximize the duration of the in vivo activity, the AHA protocol was

made more stringent by increasing the interval between inhibitor dosing and HLE challenge from 30 to 90 min.

On the basis of its oral profile, the 3-aminopyrimidone 14 was selected for further studies. The compound exhibited an oral ED_{50} of 7.5 mg/kg, and sustained activity was observed for more than 4 h following oral dosing. Oral bioavailability was excellent in three species (rat, 62%; hamster, 77%; dog, 88%). The compound was ineffective against a variety of other human proteases at concentrations 4000 times that which inhibited HLE.[18]

Pyrimidone–TFMK 14 is representative of a novel class of orally active, non-peptidic, reversible inhibitors of HLE. The modeling-assisted design of achiral, heterocyclic, dipeptide surrogates, together with the identification of appropriate molecular regions for inhibitor modification, facilitated the achievement of oral bioavailability.

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(10) (a) Wolanin, D. J. Unpublished observations from this laboratory. See also: (b) Skiles, J. W.; Fuchs, V.; Miao, C.; Sorcek, R.; Grozinger, K. G.; Maudlin, S. C.; Vitous, J.; Mui, P. W.; Jacober, S.; Chow, G.; Matteo, M.; Skoog, M.; Weldon, S.; Possanza, G.; Keirns, J.; Letts, G.; Rosenthal, A. S. Inhibition of Human Leukocyte Elastase (HLE) by N-Substituted Peptidyl Trifluoromethyl Ketones. *J. Med. Chem.* **1992**, *35*, 641–662. (c) Skiles, J. W.; Miao, C.; Sorcek, R.; Maudlin, S. C.; Mui, P. W.; Jacober, S.; Chow, G.; Skoog, M.; Weldon, S.; Possanza, G.; Keirns, J.; Letts, G.; Rosenthal, A. S. Inhibition of Human Leukocyte Elastase by N-Substituted Peptides Containing α,α-Difluorostatone Residues at P_1. *J. Med. Chem.* **1992**, *35*, 4795–4808.

(11) A recent paper has described the use of a lactam moiety as a P_3–P_2 dipeptide mimetic to generate a weak inhibitor of HLE (IC_{50} = 6.8 μM): Skiles, J. W.; Sorcek, R.; Jacober, S.; Miao, C.; Mui, P. W.; McNeil, D.; Rosenthal, A. S. Elastase Inhibitors Containing Conformationally Restricted Lactams as P_3–P_2 Dipeptide Replacements. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 773–778.

(12) Formulas derived from elemental analyses (C, H, N) for the compounds not listed in Tables 1 and 2 are as follows: **3**, $C_{15}H_{18}F_3N_3O_4 \cdot H_2O$; **9**, $C_{26}H_{22}F_3N_3O_5 \cdot H_2O$; **10**, $C_{31}H_{31}F_3N_4O_5 \cdot 0.5H_2O$; **11**, $C_{26}H_{25}F_3N_4O_5$.

(13) The K_i for the analogous *N*-acetyl-6-phenylpyridone TFMK inhibitor was 61 nM. For the synthesis of these substituted pyridones, see: (a) Bernstein, P. R.; Shaw, A.; Thomas, R. M.; Wolanin, D. J.; Warner, P. Heterocyclic Amides Having HLE Inhibitory Activity. Eur. Pat. Appl., publication number 509,769 A2, published October 21, 1992. (b) Bernstein, P. R.; Thomas, R. M.; Warner, P.; Wolanin, D. J. Lactam Dipeptides Having HLE Inhibitory Activity. Int. Pat. Appl., publication number WO 93/21212, published October 28, 1993.

(14) The K_i values of the *p*-chloro analog against HLE and BPC were 7 and 500 nM, respectively.

(15) This assay measures protection against lung damage (hemorrhage) induced by the intratracheal administration of 50 μg of HLE to anesthetized hamsters, 30 min after oral dosing of the inhibitor. For a closely related procedure, see: Fletcher, D. S.; Osigna, D. G.; Hand, K. M.; Dellea, P. S.; Ashe, B. M.; Mumford, R. A.; Davies, P.; Hagmann, W.; Finke, P. E.; Doherty, J. B.; Bonney, R. J. A Comparison of α-1-Proteinase Inhibitor Methoxysuccinyl-Ala-Ala-Pro-Val-Chloromethylketone and Specific β-Lactam Inhibitors in an Acute Model of Human Polymorphonuclear Leukocyte Elastase-induced Lung Hemorrhage in the Hamster. *Am. Rev. Respir. Dis.* **1990**, *141*, 672–677.

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(18) Compound 14 exhibited K_i selectivity ratios >4000 opposite the following human enzymes: pancreatic chymotrypsin, pancreatic trypsin, plasma thrombin, leukocyte cathepsin G, mast cell tryptase, plasma kallikrein, urine kallikrein, plasma plasmin, spleen cathepsin B, and lung angiotensin-converting enzyme. The selectivity ratios opposite PPE and BPC were 231 and 720, respectively.