Design and Synthesis of New Orally Active Nonpeptidic Inhibitors of Human Neutrophil Elastase

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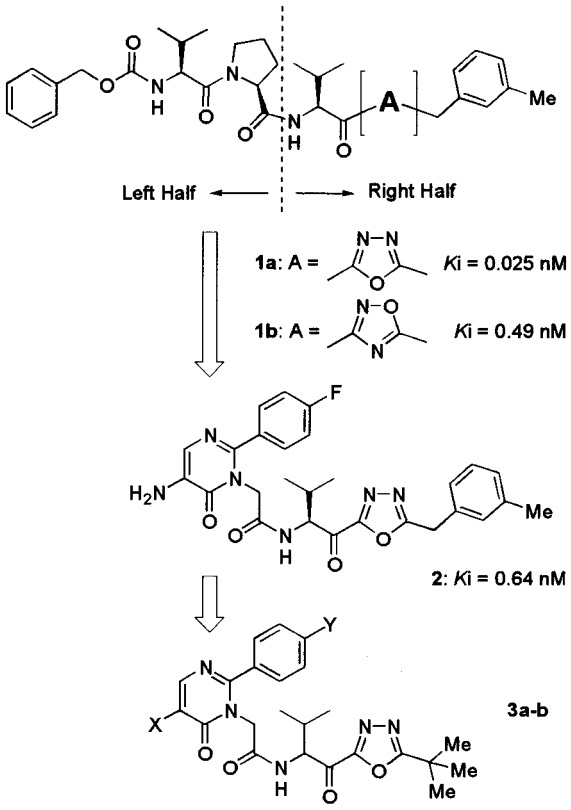
Introduction. Human neutrophil elastase (HNE) is a serine protease produced by human neutrophils in response to inflammatory stimuli.[1] Excessive production of elastase has been implicated in the etiology of various diseases such as emphysema,[2] adult respiratory distress syndrome (ARDS),[3] cystic fibrosis,[4] and rheumatoid arthritis.[5] A low-molecular-weight inhibitor of elastase would prove therapeutically useful against such pathologies.[6]

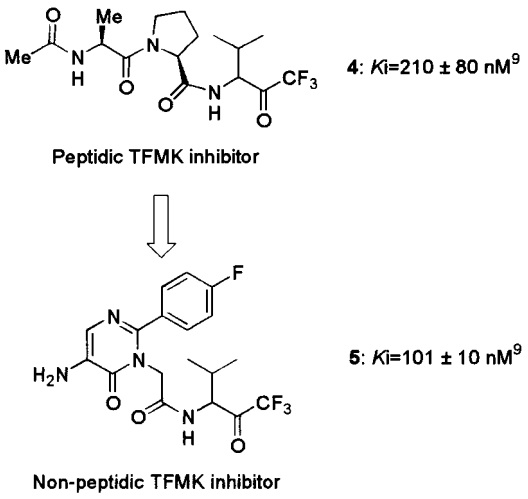
Much effort has been made to identify orally active inhibitors of HNE. Numerous synthetic inhibitors of elastase have been reported,[7] but few orally active types possessing clinical utility have been identified. In fact the only successful example to date was peptidyl trifluoromethyl ketones (TFMKs) recently reported by Zeneca.[8] We here report a highly potent orally active nonpeptidic HNE inhibitor **3a** (Scheme 1) that is currently under clinical trial (phase I).

Results and Discussion. In 1994, a peptidomimetic portion of **5** for the Ala-Pro dipeptide of the TFMK inhibitor **4** was designed by Zeneca's scientists (Scheme 2).[9] A novel nonpeptidic substitute for the Ala-Pro dipeptide portion made a series of TFMK inhibitors orally available. While the in vitro potency tended to increase by larger *N*-substituents of 5-amino-2-(4-fluorophenyl)pyrimidin-6-one, the in vivo activity was maximized in the smaller 5-amino derivative **5** with remarkable loss of the in vitro activity. A novel substitute for the TFMK portion has been needed to further improve the oral activity of TFMK inhibitors.

Scheme 1. Design of Nonpeptidic Inhibitors **3a**,**b**



Scheme 2. Design of Nonpeptidic TFMK Inhibitors



During the course of a screening program to find orally active inhibitors of HNE, peptidyl α-keto-1,3,4-oxadiazole derivative **1a**, which was derived from the corresponding 1,2,4-oxadiazole derivative **1b** ($K_i = 0.49$ nM),[10] was found to exhibit potent inhibitory activity ($K_i = 0.025$ nM)[10] against HNE (Scheme 1). While the level of in vitro activity that had been achieved with **1a** was exciting, the compound was not orally active at a dose of 30 mg/kg in an acute hemorrhagic assay according to our internal data. A number of chemical modifi-

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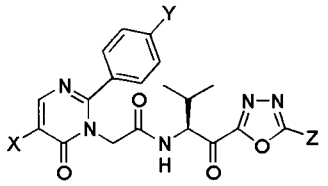
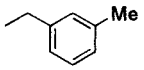
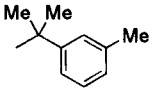
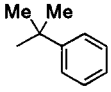
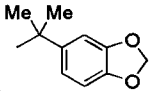
Table 1. Biological Data of Pyrimidinone α-Keto-1,3,4-oxadiazoles



Compd.	X	Y	Z	Formula[a]	*Ki* (nM)[b]	ED_{50} (mg/kg, po)[c]
2	NH_2	F		$C_{27}H_{27}FN_6O_4$	0.64[d]	13.3
6	NH_2	F		$C_{29}H_{31}FN_6O_4 \cdot 0.5H_2O$	1.37	NT[e]
7	NH_2	F		$C_{28}H_{29}FN_6O_4 \cdot H_2O$	0.52	10.0
8	NH_2	F		$C_{29}H_{29}FN_6O_6 \cdot 0.5H_2O$	1.18	NT[e]
9	NH_2	F		$C_{23}H_{27}FN_6O_4$	6.38	6.5
3b	Racemate of **9**			$C_{23}H_{27}FN_6O_4$	11.80	10.0
10	NH_2	F		$C_{23}H_{25}FN_6O_4$	15.30	9.5
11	H	F		$C_{23}H_{26}FN_5O_4 \cdot 0.7H_2O$	44.30	6.8
12	NH_2	H		$C_{23}H_{28}N_6O_4 \cdot 0.2H_2O$	3.59	6.7
13	*R*-enantiomer of **12**			$C_{23}H_{28}N_6O_4 \cdot 0.4H_2O$	29.10	6.7
3a	Racemate of **12**			$C_{23}H_{28}N_6O_4$	12.16	5.1 (1.4)[f]

[a] All compounds were analyzed for C, H, N; the results agreed to within ±0.4% of the theoretical values. [b] Inhibition of HNE-catalyzed hydrolysis of the synthetic substrate MeO-Suc-Ala-Ala-Pro-Val-pNa. [c] Inhibition of HNE-induced lung hemorrhage in hamsters ($n = 6-10$). Test compounds were administered orally (as a solution in PEG400/distilled water/ethanol = 51/33/16) 1 h before intratracheal instillation of HNE (10 U/lung). [d] See ref 10. [e] NT, not tested. [f] Test compound was administered orally as a suspension in 0.5% CMC.

cations to the left half of **1a** were made to produce a highly potent orally active inhibitor of HNE. The nonpeptidic 5-amino-2-phenylpyrimidin-6-one reported by Zeneca's scientists in 1994[9] proved to be the best modification in orally active inhibitors such as **2** and **3a**,**b** (Scheme 1).

The modification resulted in the discovery of **2** which was orally active at a dose of 13.3 mg/kg (ED_{50}). Furthermore, the *m*-methylbenzyl group on the α-keto-1,3,4-oxadiazole of the right half of **2** was amenable to a variety of synthetic modifications. A lipophilic functionality proved to be well-tolerated on the oxadiazole. This modification led us to discover a series of highly potent orally active inhibitors of HNE (Table 1). Inhibition constants (K_i) and ED_{50} values following oral dosing of representative compounds are given in Table 1. Replacement of the benzylic portion on the oxadiazoles of **2** and **6**−**8** with an aliphatic moiety produced **9**−**12** with relatively low in vitro activity, while their ED_{50} values were retained despite the decreased inhibitory activity of **9**−**12**. While removal of the 5-amino group of the 2-phenylpyrimidinone portion of **9** afforded **11** with a marked reduction of in vitro activity, the potent ED_{50} was retained. This result was speculated to be ascribed to the improved physicochemical properties of **11** such as improved Caco-2 permeability and water solubility. Removal of the *p*-fluoro group of **9** produced **12** with increased in vitro activity, while the in vivo potency (ED_{50}) was retained. More importantly, compounds **2** and **7** possessing a benzylic substituent at position 5 of their oxadiazoles exhibited nearly the same oral potency as **3a**,**b** and **9**−**12** possessing an aliphatic alkyl substituent at the corresponding position. This inconsistency between the in vitro and in vivo activities was ascribed to the presumed higher protein binding of the aromatic portions relative to the aliphatic por-

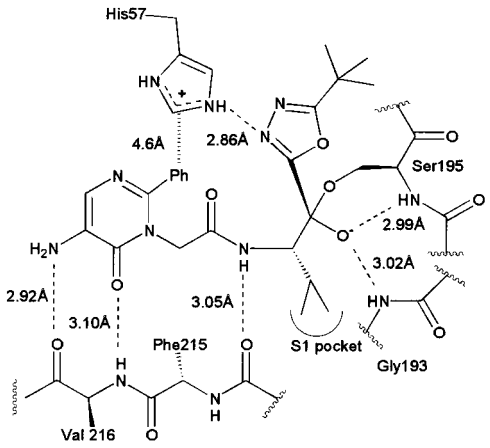


Figure 1. Schematic diagram of the binding interactions found in the crystal structure of the **3a**/PPE complex. The dashed lines represent the hydrogen bonds. The side chain of P1 (Val) was inserted into the S1 pocket of PPE. $\pi-\pi$ and/or van der Waals interaction, which is speculated to be effective for selective inhibition of enzyme, was also observed between the imidazole ring on His-57 and the phenyl ring of **3a**.

tions. Thus, the *tert*-butyl group on the oxadiazole ring proved more beneficial than the benzylic group at the corresponding position for conveying oral activity and for synthesis.

Regarding chirality, a gradual racemization of both enantiomers **12** and **13** was observed following their separate incubation in whole blood of hamster, rat, and human, because of their easily enolizable structures. As a result, all the inhibitors **3a** (*RS*-mixture), **12** (*S*-isomer), and **13** (*R*-isomer) exhibited oral activity at a dose of less than 10 mg/kg, though the in vitro activity of these three differed.

A more potent ED_{50} (1.4 mg/kg) was obtained after oral dosing of a suspension of **3a** in carboxymethyl cellulose (CMC). α-Keto-1,3,4-oxadiazole proved more effective than TFMK in improving oral activity because it presumably optimizes the physical properties of the molecule and the in vitro activity through chemical modification of the substituent on the oxadiazole.

The crystal structure of inhibitor **3a** complexed to porcine pancreatic elastase (PPE)[11] provided evidence that these inhibitors were able to bind PPE as shown in Figure 1. Formation of a stable, tetrahedral, hemiketal adduct has been demonstrated by crystallographic studies of one of our inhibitors (**3a**) complexed with the closely related enzyme, PPE. Figure 1 illustrates key enzyme−inhibitor interactions that are observed in this crystal structure. Ser-195 is covalently attached to the carbonyl of α-keto-1,3,4-oxadiazole, and the resulting oxygen anion is stabilized by hydrogen bonds to the backbone amide N−H of both Ser-195 and Gly-193 in the oxyanion hole. The isopropyl group of valine occupies the S1 specificity pocket, and $\pi-\pi$ interaction is observed between the 2-phenyl moiety of 5-aminopyrimidin-6-one and His-57. The clear hydrogen bonding observed between the position 3 nitrogen of the 1,3,4-oxadiazole and the protonated imidazole residue of His-57 of the enzyme, which is not expected in the TFMK inhibitors, serves in enzyme−inhibitor interactions. The position 5 *tert*-butyl group of the oxadiazole, which seems to protect the hydrogen-bonding network of the formed tetrahedral hemiketal adduct from the attack of a nucleophile such as water, was also speculated to be beneficial for inhibition.

Pyrimidinone α-keto-1,3,4-oxadiazole **3a** provided attractive oral profiles: for example, **3a** was active at 1.4 mg/kg (ED_{50}), while TFMK **5** was active at 7.5 mg/kg (ED_{50}) in an animal model.[9] On the basis of its oral profile, potent K_i value, and relative ease of synthesis, compound **3a** (ONO-6818) was selected for further evaluation. The compound exhibited a highly potent oral activity (ED_{50}: 1.4 mg/kg) which lasted for more than 8 h. Oral bioavailability was excellent in three species (rat, 51%; dog, 31%; monkey, 18%).

Compound **3a** was ineffective against a variety of proteases such as trypsin, proteinase 3, pancreatic elastase, *Pseudomonas aeruginosa* elastase, plasmin, thrombin, type I collagenase, cathepsin G, and murine macrophage elastase at a concentration 100 times that which inhibited HNE.

The 5-aminopyrimidinone α-keto-1,3,4-oxadiazole **3a** (ONO-6818) is representative of orally active nonpeptidic reversible inhibitors of HNE. The newly discovered heterocyclic surrogate for the TFMK moiety contributed to the identification of an excellent oral profile. Further details[12] will be reported in a following full paper.

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(11) Compound **3a** showed 100-fold less activity toward PPE.
(12) Chemistry will be introduced in a following full paper which has been submitted to this Journal.

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