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N-Acyl and *N*-sulfonyloxazolidine-2,4-diones are pseudo-irreversible inhibitors of serine proteases

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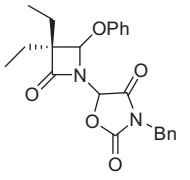
ABSTRACT

The synthesis, inhibitory activity and mode of action of oxazolidine-2,4-diones against porcine pancreatic elastase, here used as a model for human neutrophil elastase, are reported. The nature of N-substitution at the oxazolidine-2,4-dione scaffold has large effect on the inhibitory potency against elastase. *N*-Acyl and *N*-sulfonyloxazolidine-2,4-diones emerged as potent pseudo-irreversible inhibitors, displaying high second-order rate constants for PPE inactivation. The title compounds were also shown to be potent inhibitors of human neutrophil elastase (HNE) and proteinase-3, and weak inhibitors of human cathepsin G. The results herein presented show that the oxazolidine-2,4-diones represent a new promising class of serine protease inhibitors.

Human neutrophil elastase (HNE), a serine protease of the chymotrypsin superfamily stored in the primary azurophilic granules of polymorphonuclear neutrophils, is a major etiologic factor in chronic inflammatory diseases such as acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF).[1–4] During these inflammatory processes, neutrophils release an excess of HNE to the extracellular medium where, in absence of appropriated levels of endogenous inhibitors, can hydrolyze a variety of matrix proteins such as elastin and collagen.[5,6] Hence, HNE inhibition has been recognized as a valid therapeutic approach to protect the lungs from HNE-mediated tissue damage but also to control over exuberant inflammatory responses.[7]

In our previous work, the unexpected synthesis of azetidin-2-ones containing a oxazolidin-2,4-dione moiety, for example **1**, drew our attention to the potential of oxazolidine-2,4-diones as elastase inhibitors.[8] Structurally, oxazolidine-2,4-diones are isosteric to succinimide, **2**, and 1,2,5-thiadiazolin-3-one 1,1-dioxide, **3**, derivatives, which are two well-known classes of HNE inhibitors.[9–12] The first step in the mechanism of HNE inhibition by **2** and **3** involves nucleophilic attack of the active site serine residue (Ser-195) to the carbonyl carbon atom at the five-membered ring to generate a tetrahedral intermediate that collapses via ring-opening to give a relatively stable acyl enzyme. The departure of a leaving group (a sulfonate for **2** and a carboxylate for **3**) then can trigger a suicide-type inactivation mechanism in the active site of HNE.[9,13,14]

The rate of serine acylation is controlled by the molecular recognition by the target enzyme and intrinsic reactivity of the inhibitor. Improving the rate of serine acylation by increasing the



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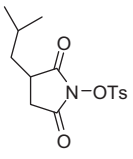

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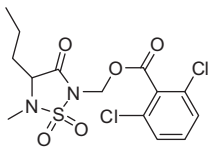

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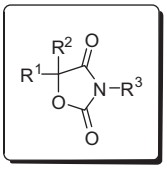

4

R^1, R^2 = H, Me, Ph
R^3 = Ar, COAr, SO_2Ar

intrinsic chemical reactivity of acylating agents such as β- and γ-lactams has been a successful strategy to design more potent elastase inhibitors.[15–17] Usually, this has been performed by mod-

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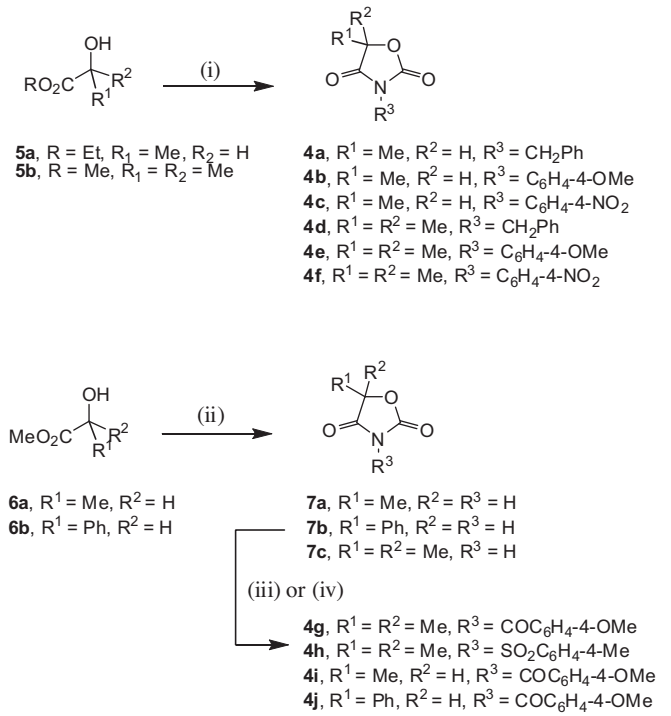

Scheme 1. Synthesis of oxazolidine-2,4-diones **4a–j**. Reagents and conditions: (i) R^3NCO, DCM or THF, TEA, reflux; (ii) urea, MeONa/MeOH; (iii) **4g**, **4i** and **4j**: R^3COCl, THF, TEA, reflux; (iv) **4h**: R^3SO_2Cl, Na, THF, reflux.

ulating the electrophilic nature of the acyl center and the leaving group ability of the group that results from the collapse of the tetrahedral intermediate and lactam ring-opening.

To assess the potential of oxazolidine-2,4-diones as elastase inhibitors, we synthesized derivatives, **4**, bearing *N*-aryl-, *N*-benzyl, *N*-acyl- and *N*-sulfonyl moieties, which were selected to convey intrinsic chemical reactivity required for a fast acylation of the Ser-195. In this study, compounds **4** were initially tested in vitro towards porcine pancreatic elastase (PPE), a model enzyme that shares 40% of homology and the catalytic triad with HNE.[4] The enzymatic recognition by S_1 subsite may be achieved by appending methyl groups to C-5 of the oxazolidine-2,4-dione scaffold, as PPE and HNE prefer small lipophilic groups for molecular recognition.[18,19]

Oxazolidine-2,4-diones **4a–j** were synthesized as illustrated in Scheme 1. The reflux of 2-hydroxypropanoates **5** with the appropriate isocyanate in anhydrous DCM or THF and in the presence of triethylamine afforded directly the *N*-benzyl- and *N*-aryl-oxazolidine-2,4-diones **4a–f**. *N*-Acyl- and *N*-sulfonyloxazolidine-diones **4g–j** were prepared starting from 2-hydroxy esters **6**, which were converted to the oxazolidine-2,4-diones **7** by reaction with urea and sodium methoxide. Reaction of **7** with 4-methoxybenzoyl chloride or 4-methylbenzene-sulfonyl chloride in anhydrous THF containing triethylamine or sodium gave the *N*-acyl- and *N*-sulfonyloxazolidinediones **4g–j**.

The inhibitory activity of compounds **4a–j** towards PPE was determined using Kitz and Wilson's pre-incubation method.[20] No inhibition of the enzymatic activity was observed in the incubation of 600-fold molar excess of **4a** or **4b**, and 300-fold molar excess of **4c**, **4e** and **4f** with PPE. In contrast, **4d** and **4g–j** inhibited PPE in a time- and concentration-dependent manner. For example, exposure of PPE to 100-fold molar excess of compounds **4g** or **4h** resulted in a very rapid loss of enzymatic activity which reached ca. 96% and 92% of inhibition, respectively. However, the enzymatic activity was almost completely recovered within 72 min when the

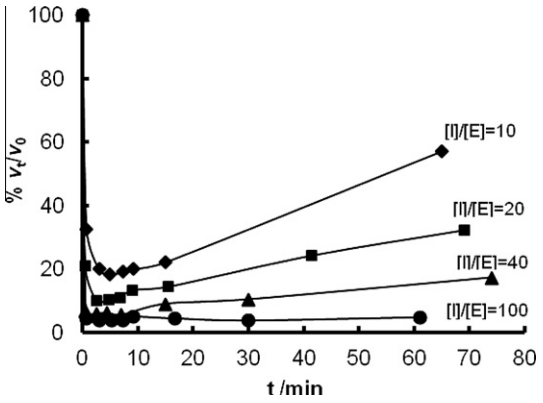

Figure 1. Time-dependent loss of enzymatic activity. Porcine pancreatic elastase (5 μM) was incubated with and various amounts of **4g** (50–500 μM) in 0.1 M HEPES buffer, pH 7.2. Aliquots were removed periodically and assayed for enzymatic activity using *N*-Suc-(L-Ala)$_3$-*p*-nitroanilide.

Table 1
Half-lives in pH 7.4 phosphate buffer at 37 °C, second-order rate constant for inactivation, k_{obs}/[I], second-order rate constant for the alkaline hydrolysis k_{OH^-}, and enzyme-rate-enhancement factor for oxazolidine-2,4-diones **4a–j**

Compound	$t_{½}$[a] (min)	k_{obs}/[I]/$M^{-1}s^{-1}$	$k^{b}_{OH^-}$/$M^{-1}s^{-1}$	EREF[c]
4a	ND[d]	NI[e]	ND	—
4b	ND	NI	2.14	—
4c	38.7	NI	ND	—
4d	ND	3.02	ND	—
4e	ND	NI	ND	—
4f	ND	NI	ND	—
4g	16.7	467	8.75	53
4h	6.86	255	92.2	3
4i	ND	133	ND	—
4j	ND	1320	ND	—

[a] pH 7.4 phosphate buffer, T = 37 °C.
[b] Borate buffer containing 20% (v/v) of acetonitrile, T = 25 °C.
[c] Enzyme-rate-enhancement factor, EREF = k_{obs}/k_{OH^-}[I].
[d] ND, not determined.
[e] NI, no inhibition.

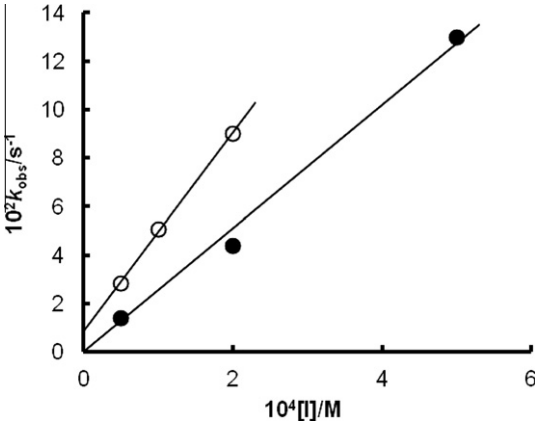

Figure 2. Plots of first-order rate constants, k_{obs}, for the inactivation of PPE versus the concentration of inhibitors **4g** (●) and **4h** (○).

inhibition was carried out by **4h**, suggesting that an instable acyl–enzyme complex was formed, whereas no recovery of activity was observed after 61 min of PPE inhibition by **4g** (Fig. 1). The inhibitory potency of compounds **4d** and **4g–j** against PPE was expressed as k_{obs}/[I] (second-order rate constant for inactivation) and is listed in Table 1. These values were determined from the plots of first-or-