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1,3-Oxazino[4,5-b]indole-2,4-(1*H*,9*H*)-diones and 5,6-Dimethylpyrrolo-[2,3-d]-1,3-oxazin-2,4-(1*H*,7*H*)-diones as Serine Protease Inhibitors

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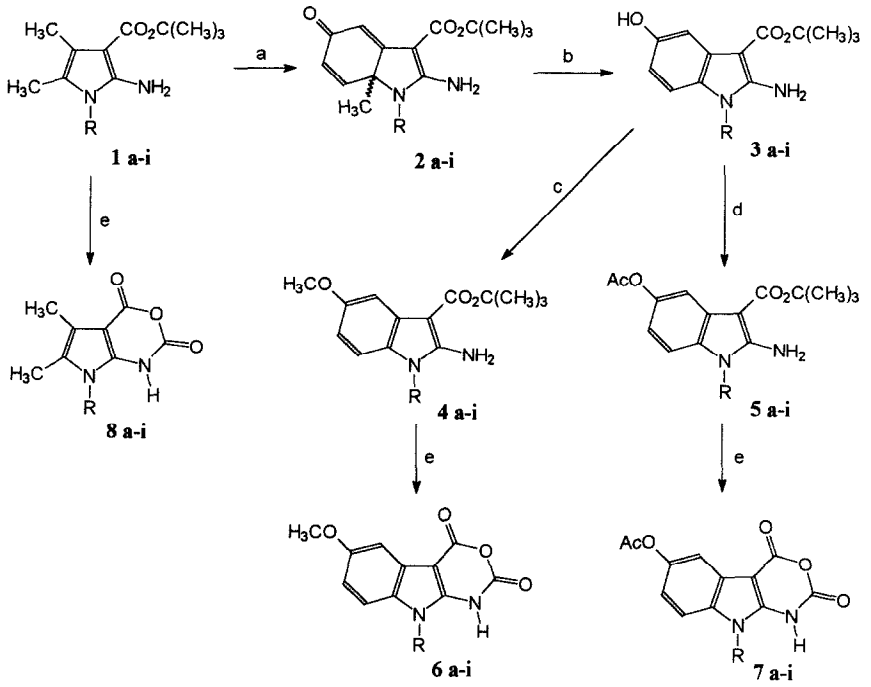
Abstract 9-Substituted-1,3-oxazino[4,5-b]indole-2,4-(1*H*,9*H*)-diones and 7-substituted-5,6-dimethylpyrrolo[2,3-d]-1,3-oxazin-2,4-(1*H*,7*H*)-diones are synthesized from indolo- or pyrrolo-β-enamino *t*-butyl esters in moderate yield. Certain compounds are found to inhibit human leukocyte elastase (HLE) and chymotrypsin selectively.

Human leukocyte elastase (HLE, EC 3.4.21.37) is a strongly basic serine protease found in the azurophilic granules of human polymorphonuclear leukocytes.[1] It has been implicated in diverse pathological states, e.g., pulmonary emphysema,[2] adult respiratory distress syndrome (ARDS)[3] and rheumatoid arthritis.[4] Perhaps best understood is its role in emphysema in which it attacks the fibrous protein elastin, a major component of lung tissue, resulting in the connective tissue degeneration seen in that disease. The proteinase-antiproteinase imbalance postulate[5] suggests that increased activity of HLE and/or decreased activity of α-1 proteinase inhibitor (α-1 PI, α-1 antitrypsin) results in increased proteolysis, especially elastolysis. In fact, persons with a genetic deficiency of α-1 PI typically develop emphysema at an early age. Cigarette smoke, a major causative agent worldwide in the development of chronic obstructive pulmonary disease (COPD) and emphysema, has been shown to inactivate α-1 PI *in vitro*[6] and may cause a localized α-1 PI deficiency in the lungs of smokers. A specific, biologically stable and potent inhibitor of HLE would have distinct clinical advantages.[7]

Many different classes of heterocycles have been shown to inhibit elastase including, chloroisocoumarins,[8] benzoxazinones,[9] hydantoins,[10] cephalosporins[11] and anthraquinones.[12] Most recently several 1,3-oxazino[6,5-b]indole-2,4-(3*H*,9*H*)-diones and 2*H*-1,3,5-oxadiazino[3,2-a]indole-2,4-(3*H*)-diones were found to be inhibitors of tissue plasminogen activase but to lack

adequate selectivity.[13] We have recently developed new technology for the conversion of heterocyclic β-enamino *t*-butyl esters to 1,3-oxazin-2,4-diones (Scheme I). The 2-amino-3-*t*-butoxycarbonylpyrroles **1 a-i** may be converted to the enone **2 a-i** by means of a facile cycloaddition of ethyl propiolate in a protic solvent.[14] Reductive aromatization of **2 a-i** to the 2-amino-3-*t*-butoxycarbonyl-5-hydroxyindole **3 a-i** is accomplished with Zn in pyridine and a trace of water.[15] The 5-hydroxyindoles **3 a-i** were *O*-methylated or *O*-acetylated with dimethyl sulfate or acetic anhydride, respectively. Oxazin-2,4-dione formation **6, 7, 8 a-i** then takes place in high yield on treatment with Triphosgene® without base.[16] We chose to test these compounds **6, 7, 8 a-i** against human leukocyte elastase as well as porcine pancreatic elastase and chymotrypsin, serine proteases with sequence homologies and catalytic mechanisms similar to those of HLE.

Scheme 1



(a) Ethyl propiolate, ethanol, reflux. (b) Zn, pyridine/H_2O, reflux. (c) $(CH_3O)_2SO_2$, NaH, THF, reflux. (d) Ac_2O, r.t. (e) $(Cl_3CO)_2CO$, CH_2Cl_2, reflux.

Table 1

Compound	R	IC_{50} (μM) PPE	HLE	Chymotrypsin
6a	n-C_3H_7	NI	>1000	>1000
6b	n-C_4H_9	NI	>1000	486
6c	c-C_6H_{11}	NI	>1000	>1000
6d	$CH_2CH_2OCH_3$	NI	>1000	NI
6e	$CH_2CH(OCH_3)_2$	NI	>1000	>1000
6f	$CH_2C_6H_5$	NI	>1000	40
6g	$CH_2CH_2C_6H_5$	NI	>1000	>1000
6h	$CH_2CH_2CH_2C_6H_5$	NI	989	>1000
6i	CH_2(2-C_5H_4N)	NI	>1000	512
7a	n-C_3H_7	NI	665	>1000
7b	n-C_4H_9	>1000	810	>1000
7c	c-C_6H_{11}	NI	454	>1000
7d	$CH_2CH_2OCH_3$	NI	>1000	NI
7e	$CH_2CH(OCH_3)_2$	NI	742	>1000
7f	$CH_2C_6H_5$	NI	75	>1000
7g	$CH_2CH_2C_6H_5$	833	92	>1000
7h	$CH_2CH_2CH_2C_6H_5$	>1000	305	>1000
7i	CH_2(2-C_5H_4N)	NI	292	>1000
8a	n-C_3H_7	NI	NI	NI
8b	n-C_4H_9	NI	>1000	>1000
8c	c-C_6H_{11}	NI	>1000	510
8d	$CH_2CH_2OCH_3$	NI	NI	NI
8e	$CH_2CH(OCH_3)_2$	NI	NI	NI
8f	$CH_2C_6H_5$	NI	NI	740
8g	$CH_2CH_2C_6H_5$	NI	NI	105
8h	$CH_2CH_2CH_2C_6H_5$	NI	895	422
8i	CH_2(2-C_5H_4N)	NI	NI	>1000

The inhibitors (**6**, **7**, **8 a-i**; 833 μM) were incubated with HLE, PPE, or chymotrypsin in a 0.1 M HEPES, 0.5 M NaCl, pH 7.5 buffer at room temperature for 30 min and residual enzymatic activities were measured using MeO-Suc-Ala-Ala-Pro-Val-*p*NA (416 μM), Suc-Ala-Ala-Ala-*p*NA (416 μM), Suc-Ala-Ala-Pro-Phe-*p*NA (416 μM) as substrates for HLE, PPE, and chymotrypsin, respectively.[17] If inhibition was observed at 833 μM, the IC_{50} values were obtained by measuring

residual enzyme activity at various inhibitor concentrations (Table 1). For compounds **6**, **7 a-i**, the best inhibitors of HLE are **7f** and **7g** which contain 6-acetoxy and 9-benzyl or 9-phenylethyl substituents (IC_{50} = 75μM and 92 μM, respectively). Compounds **7 a-i** with a 6-acetoxy substituent are better HLE inhibitors than the corresponding 6-methoxy derivatives (**6 a-i**). Compounds **8 a-i** did not inhibit HLE. The active site of HLE is much more hydrophobic than that of PPE and the 9-substituent (benzyl or phenylethyl) may be interacting with hydrophobic residues in HLE's active site. Compounds **6**, **7**, and **8 a-i** did not effectively inhibit PPE. The best chymotrypsin inhibitor is **6f** (IC_{50} = 40 μM) which contains 6-methoxy and 9-benzyl groups. Compounds **6b**, **6f**, and **6i** with a 6-methoxy group inhibited chymotrypsin more potently than the corresponding derivatives with a 6-acetoxy moiety (**7b**, **7f**, **7i**). Among **8 a-i**, only compounds containing large hydrophobic groups such as **8c**, **f**, **g**, **h** showed some inhibition towards chymotrypsin, which is consistent with chymotrypsin's specificity. The mechanism of inhibition probably involves acylation of the active site serine residue as has been observed with other types of heterocyclic inhibitors.[8,9,10,11] including several isatoic anhydride derivatives. [18,19] To confirm this mechanism, we studied the time dependence of the inhibition reactions with a few of the better inhibitors and their reactivations with hydroxylamine. Chymotrypsin was inhibited by both **6f** and **8g** in a time dependent manner and the kinetics are shown in Figure 1. The second order inhibition constants ($k_{obs}/[I]$) were 4.6 and 0.3 $M^{-1}s^{-1}$ at an inhibitor concentration of 420 μM. Chymotrypsin inhibited by **6f** or **8g** was quite stable and did not regain any enzymatic activity after addition of NH_2OH (0.3 or 0.45 M) and incubation at room temperature for one day. HLE was also irreversibly inhibited by **7f** or **7g** with k_{obs} /[I] values of 22.3 and 5.3 $M^{-1}s^{-1}$ at 139 and 420 μM, respectively. However, the inhibited HLE underwent partial reactivation during the course of the assays and still retained 20-40% activity after incubation with inhibitor. The addition of 0.3-0.45 M NH_2OH to the inhibited HLE solution resulted in the recovery of 70-100% of enzymatic activity in a few min. These results are consistent with the formation of one or more acyl enzyme derivatives upon reaction of HLE with **7f** and **7g**, all of which regain activity upon treatment with hydroxylamine. In the case of chymotrypsin inhibited by **6f** or **8g**, a NH_2OH non-reactivatable acyl enzyme derivative is probably formed. It is likely that the acyl group attached to the active site serine is not accessible to NH_2OH. Alternately, it is possible that the inhibitors **6f** and **8g** have reacted with other residues of chymotrypsin.

Figure 1

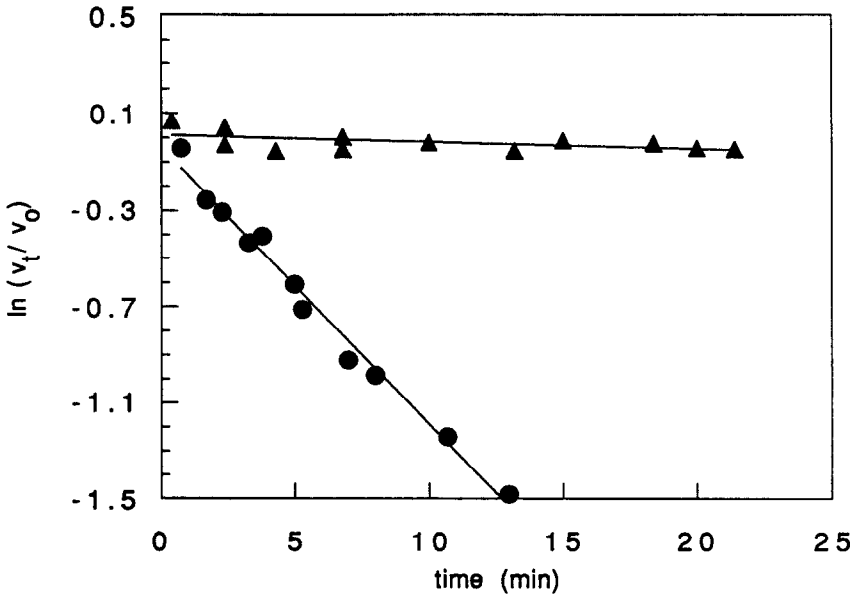


Figure 1. Inhibition of chymotrypsin by **6f** at an inhibitor concentration of 420 μM. Chymotrypsin (5 μM, 50 μL) was incubated with the inhibitor solution (5 mM, 50 μL) or Me_2SO (control) in 0.5 mL of 0.1 HEPES, 0.5 M NaCl, pH 7.5 buffer. An aliquot (40 mL) was withdrawn at various time intervals and added to a Suc-Ala-Ala-Pro-*p*NA assay mixture to monitor the residual enzyme activity. The circles represent the reaction mixture containing **6f**, the triangles represent the reaction mixture containing only Me_2SO. The initial rate at reaction time t min is v_t and v_0 is the reaction rate at time zero.

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