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Dual-Action Inhibitors of Proteolytic Enzymes: Potential Therapeutic Agents for Cystic Fibrosis and Related Ailments

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Abstract—The design, synthesis and *in vitro* inhibitory activity toward human leukocyte elastase of a series of dual action saccharin derivatives are described.

Introduction

Cystic fibrosis (CF) is a genetic disease associated with a high incidence of morbidity and mortality.[1] Recent studies have led to the identification of the defective gene and the protein (termed cystic fibrosis transmembrane regulator, CFTR), encoded by this gene.[2,3] CFTR has been shown to function as a chloride ion channel that modulates the permeability of chloride ion in response to elevations of intracellular cAMP.[4]

Several lines of evidence suggest that neutrophil-derived proteolytic enzymes, particularly elastase, play a significant role in the pathophysiology of CF. For instance, the progressive damage to lung tissue observed in CF is likely due to the presence of free elastase, a serine proteinase that is known to degrade elastin and other matrix proteins.[5-7] Equally important is the observation that elastase degrades C3b receptors, thereby contributing to the inability of CF patients to eradicate *P. aeruginosa* from their lungs.[8] Elastase and cathepsin G play an important role in the production of increased and abnormal secretions associated with CF.[9] Lastly, elastase induces human bronchial epithelial cells to release interleukin-8 (IL-8), a potent neutrophil chemoattractant.[10]

In addition to the release of proteolytic enzymes, the influx of neutrophils involves the release of mediators of inflammation and endogenous oxidants.[11] These oxidants have been shown to inactivate alpha-1-proteinase inhibitor (alpha-1-PI) and secretory leukoprotease inhibitor (SLPI), the physiological inhibitors of elastase.[12,13]

Strategies that seek to counteract the damaging effects of the aforementioned processes may be effective in the management of CF. This paper describes the results of synthetic and biochemical studies using some saccharin derivatives having a dual mode of action.

Chemistry

Compounds **1–7** (Table 1) were synthesized by reacting *N*-chloromethyl saccharin with the appropriate agent in acetonitrile. The physical constants and spectral data of compounds **1–6** are listed in Table 2.

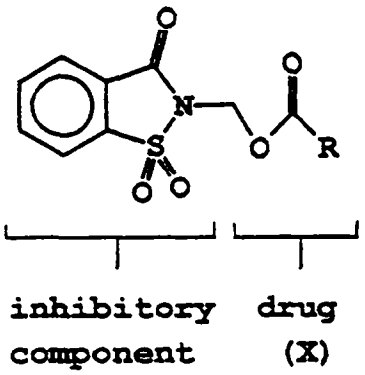
Biochemical Studies

The enzyme assays and inhibition studies were carried out as described in detail elsewhere.[14,15] The apparent second order inactivation rate constants, $k_{obs}/[I]$ $M^{-1}s^{-1}$ are listed in Table 1.

Results and Discussion

Rationale underlying the design of inhibitor I

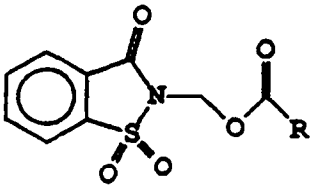
The genetic defect in CF leads to various clinical manifestations arising from the interplay of many mediators associated with the inflammatory response, the influx of neutrophils and the damaging effects of proteolytic and oxidative processes. We reasoned that agents which inhibit the deleterious action of neutrophil-derived proteolytic enzymes and capable of releasing a second bioactive molecule possessing anti-inflammatory, antibacterial or antioxidant activity during the inactivation process may have superior properties as therapeutic agents The soundness of the biochemical rationale underlying the design of **I** is supported by recent literature reports.[10,16]

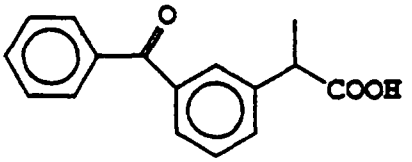
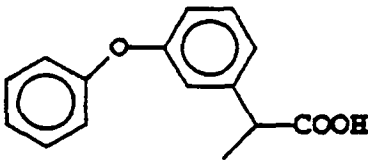
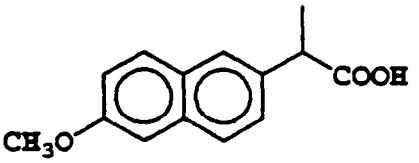
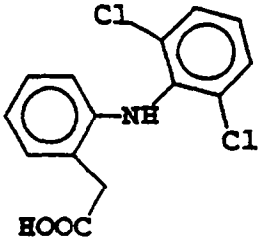
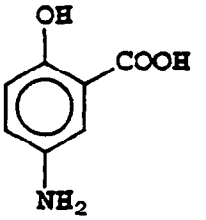
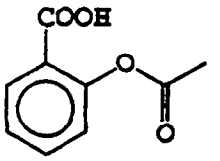


I

Based on preliminary studies related to the inhibition of HLE by saccharin derivatives,[17] it was anticipated that the attachment of an appropriate drug (X) to the saccharin nucleus would yield a two-component entity **I** capable of inactivating elastase according to the mechanism shown in Scheme I. This mechanism entails an enzyme-induced ring opening via acylation of the active site serine, followed by the release of a bioactive component (X).

Table 1.

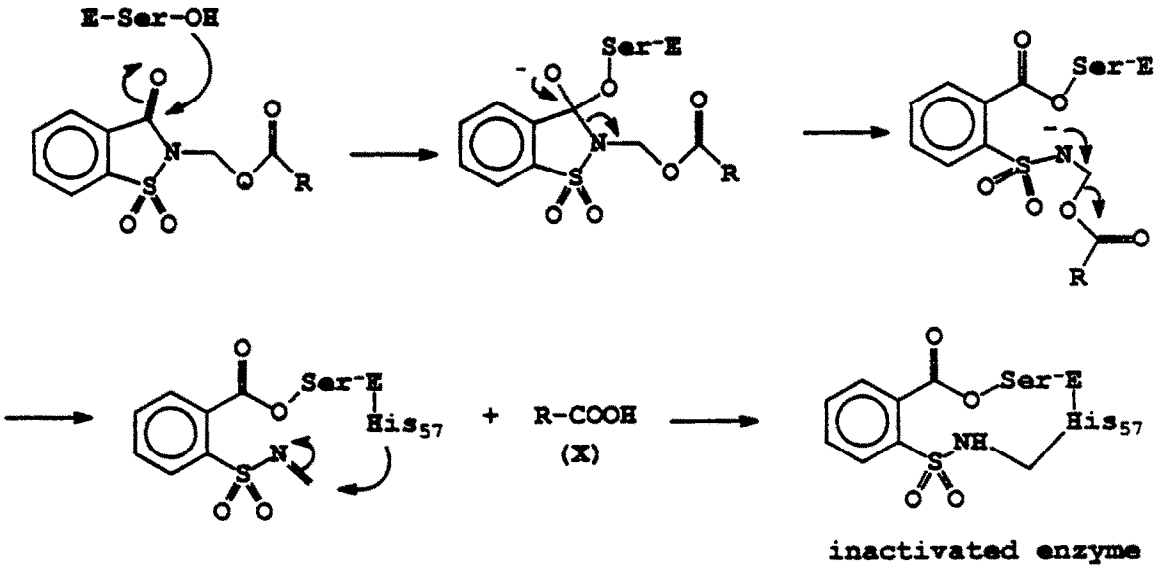


	R-COOH[a]	
1	 (RS) Ketoprofen 3300 (30)	
2	 (RS) Fenoprofen 1475 (20)	
3	 (S) Naproxen 1800 (5)	
4		 Diclofenac 430 (30)
5		 5-ASA 1260 (40)
6		 3480 (30)
7		(RS) Ibuprofen[b] 180 (20)

[a] $k_{obs}/[I]$ $M^{-1}s^{-1}$ values (SD).
[b] Taken from Ref. 17.

Table 2. Physical constants and spectral data of inhibitors

Compound	MP °C	NMR ppm	MF (anal)
1	124-125	1.6(d,3H), 3.9(q,1H), 5.9.(dd,2H), 7.4-8.0(m,12H), 8.1(d,1H)	$C_{24}H_{19}NSO_6$ (C,H,N)
2	96-97	1.5(d,3H), 3.7-3.8(q,1H), 5.75(d,1H), 5.9(d,1H), 6.85(m,1H), 7.0-7.1(m,5H), 7.3(m,3H), 7.8-8.0(m,3H), 8.1(d,1H)	$C_{23}H_{19}NO_6S$ (C,H,N)
3	132-134	1.6(d,3H), 3.9(s,4H), 5.9(dd,2H), 7.1-7.2(m,2H), 7.4 (d,1H), 7.7-7.8(m,3H), 7.8-7.9(m,3H),8.1(d,1H)	$C_{22}H_{19}NSO_6$ (C,H,N)
4	135-136	3.8(5,2H), 5.8(5,2H), 6.45(d,1H), 6.55(s,1H), 6.9(m,2H), 7.05(m,1H), 7.15(m,1H), 7.25(d,2H), 7.7-7.9(m,3H), 8.0(d,1H)	$C_{22}H_{16}N_2O_5Cl_2S$ (C,H,N)
5	160-161	4.85(s,2H), 6.1(s,2H), 6.75(d,1H), 6.85(dd,1H), 7.0(d,1H), 8.1(m,2H), 8.2(d,1H), 8.4(d,1H), 9.45(s,1H)	$C_{15}H_{12}N_2O_6S$ (C,H,N)
6	113-114	2.4(s,3H), 6.05(s,2H), 7.1(dd,1H), 7.3(dd,1H), 7.6(m,1H), 7.8-8.0(m,3H), 8.04(dd,1H), 8.15(d,1H)	$C_{17}H_{13}NO_7S$ (C,H,N)



Scheme I.

Structure–activity relationship studies

The inhibition of HLE by the compounds listed in Table 1 was time-dependent and followed pseudo first-order kinetics. For example, incubation of compound **4** with HLE led to progressive loss of enzymatic activity with very little regain in activity after 24 h (Figure 1(A)). HLE was rapidly acylated by the rest of the compounds and regained full activity slowly (the half lives of reactivation ranged between 12–14 h) (Figure 1(B)). With the exception of **7**, these compounds were found to be very efficient in inactivating HLE. The ketoprofen and aspirin derivatives (**1** and **6**) had the highest potency. There was no obvious correlation between potency, as reflected by the magnitude of the bimolecular rate constant, $k_{obs}/[I]$ $M^{-1}s^{-1}$, and the structure of (X). However, as is evident from Figure 1, the nature of X does effect the deacylation rate constant. The effect of chirality on inhibitory activity was not investigated in the present study.

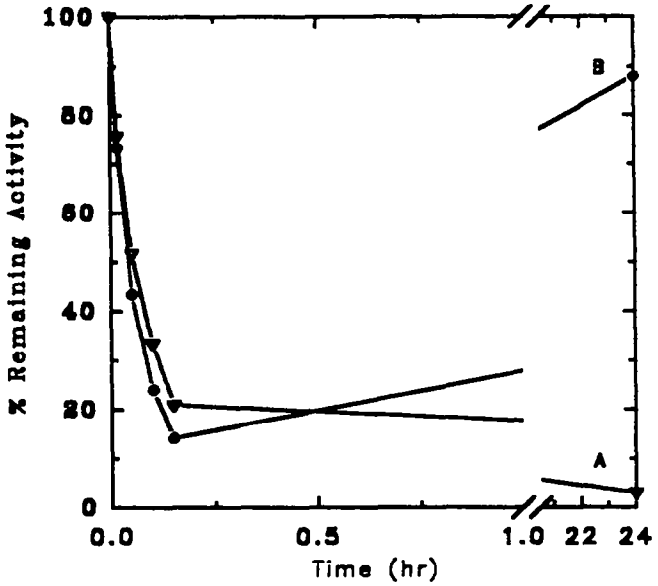


Figure 1. Time dependent loss of enzymatic activity. Human leukocyte elastase (302 nM) was incubated with compound **4** (0.302 μM) (A) or compound **5** (0.302 μM) (B) in HEPES buffer, pH 7.2, 0.5 M NaCl and 1% DMSO. Aliquots were withdrawn at various time intervals and assayed for remaining enzyme activity as previously described.[15]

In summary, this study describes the design, synthesis and preliminary *in vitro* evaluation of the inhibitory activity of a series of dual action inhibitors of human leukocyte elastase.

Experimental Section

Melting points were recorded on a Mel-Temp apparatus. and are uncorrected. The infrared and NMR spectra of the synthesized compounds were recorded on a Perkin-Elmer 1330 infrared spectrophotometer and a Varian XL-300 NMR spectrometer, respectively. A Gilford UV/VIS spectrophotometer was used in the enzyme assays and inhibition studies. Human leukocyte elastase was purchased from Elastin Products Co., Owensville, St Louis. Human leukocyte cathepsin G was obtained from Athens Research and Technology Co., Athens, GA. Methoxysuccinyl Ala-Ala-Pro-Phe *p*-nitroanilide, (*RS*)-ketoprofen, (*RS*)-ibuprofen, (*RS*)-fenoprofen, (*S*)-naproxen and diclofenac were purchased from Sigma Chemicals Co., St Louis, MO.

General synthetic procedure

A solution of *N*-chloromethyl saccharin (4 mmol), carboxylic acid (4 mmol) and triethylamine (4.8 mmol) in 15 ml acetonitrile was refluxed for 3 h The reaction mixture was cooled to room temperature and filtered. The filtrate was diluted with ethyl acetate (30 mL), washed with water and dried. Removal of the solvent *in vacuo* yielded a crude product that was purified on a chromatotron plate. The physical and spectral data of all synthesized compounds are listed in Table 2.

Acknowledgement

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