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A BENZISOTHIAZOLONE CLASS OF POTENT, SELECTIVE MECHANISM-BASED INHIBITORS OF HUMAN LEUKOCYTE ELASTASE.

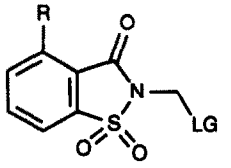
Dennis J. Hlasta,* Malcolm R. Bell, Neil W. Boaz,† John J. Court, Ranjit C. Desai, Catherine A. Franke,† Albert J. Mura,† Chakrapani Subramanyam, and Richard P. Dunlap†

Department of Medicinal Chemistry, Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Inc, 1250 South Collegeville Road, Collegeville, Pennsylvania 19426

†*Research Laboratories, Eastman Kodak Company, Rochester, New York 14650*

Abstract. A new type of mechanism-based inhibitor of human leukocyte elastase (HLE) is described that are designed to inhibit HLE by a suicide route to form an inhibitor-enzyme complex by cross linking the enzyme active site. A mechanism of HLE inhibition is proposed and was used to design analogues with improved potency. The structure-activity relationships described in this paper are consistent with the proposed mechanism of HLE inhibition, and led to WIN 62785 (**12**), the most potent compound in this series with a $K_i^* = 0.3$ nM.

Human leukocyte elastase (HLE) has been proposed to be a primary mediator of pulmonary emphysema.[1] In emphysematous patients neutrophils at the inflammation sites release a mixture of proteases, including HLE, cathepsin G, collagenase and oxidases. The endogenous regulatory protein, α_1-protease inhibitor, normally inactivates the free HLE that may be present in the lung. An imbalance between HLE and the endogenous regulatory protein is postulated to occur in emphysema, where the deficiency of the α_1-protease inhibitor leads to elastin connective tissue destruction in the lung by HLE. This hypothesis is supported by the observation that individuals with genetic α_1-protease inhibitor deficiency have less than 20% of normal inhibitor activity and a majority of these individuals develop emphysema.



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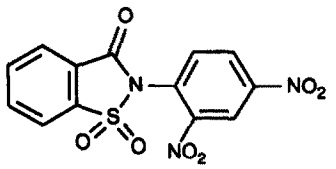

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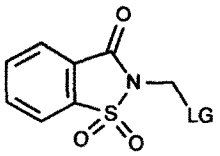
Many types of HLE inhibitors have been reported in the literature[2] which include mechanism-based inhibitors,[3-5] alternate substrate inhibitors,[6] and transition state analog inhibitors,[7] all of which bind covalently to HLE. We have discovered a class of potent, mechanism-based inhibitors of this serine protease, which may lead to a therapeutic agent for the treatment of emphysema and inflammatory pulmonary diseases. This paper describes a series of benzisothiazolone inhibitors **1** and a proposed mechanism by which these compounds inhibit HLE.[8,9] These compounds were designed to inhibit HLE by a mechanism-dependent suicide route to form an inhibitor-enzyme complex by cross linking the enzyme active site Ser^{195} and His^{57}. Acylative and alternate substrate inhibitors such as compound **2** with the benzisothiazolone nucleus have been reported.[10,11] These inhibitors

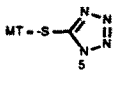
were proposed to acylate the active site Ser^{195}, but lack the second activation step of a mechanism-based inhibitor.[12] Micromolar concentrations of **2**[11] were required for HLE inhibition compared to nanomolar concentrations for our mechanism-based inhibitors (see Table 1, **7** K_i^* = 15 nM).

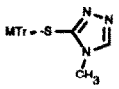
The proposed mechanism of HLE inhibition by the compounds **1** is outlined in Scheme I. After Ser^{195} of HLE adds to the carbonyl causing ring scission, the leaving group (LG) is eliminated resulting in the formation of a reactive intermediate (see Scheme I) which bears an electrophilic center in the sulfonyl imine group. Addition of His^{57} to the sulfonyl imine gives the inhibitor-enzyme complex. After forming the inhibited complex, by design these compounds have a mechanism for enzyme reactivation through a pathway different from the inactivation pathway. Since the mechanisms of inactivation and reactivation are different, we predicted that substituent effects on the benzisothiazolone ring would *independently affect the inactivation and reactivation rates*. Also, the reactivation rates should remain constant for inhibitors that possess the same nucleus and which vary only in the leaving group, since these inhibitors would all form the same inhibitor-enzyme complex. The enzyme kinetic data and the structure-activity relationships discussed below are consistent with these predictions and the proposed mechanism of inhibition.

Table 1. Leaving Group Modification Effects on HLE Inhibition.



Cmpd	LG =	HLE[a] k_{inact} ($M^{-1}sec^{-1}$)	HLE[a] k_{react} (sec^{-1})	HLE[a] K_i^* (nM)
3	OAc	630	0.000064	102
4	Cl	44,000	0.000079	1.8
5	MT-5-CH_3	1,000	0.000081	81
6	MT-5-cyclohexyl	2,500	0.000080	32
7	MT-5-C_6H_5	5,600	0.000084	15
8	MTr	190	0.000047	246



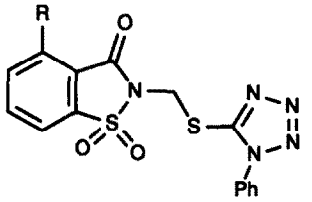


[a] The potency of inhibition is expressed as an apparent binding constant, K_i^*, where $K_i^* = k_{react}/k_{inact}$. The rates and binding constants were reproducible to within ±10%.

The series of inhibitors shown in Table 1 have the same nucleus, but differ structurally based on the leaving group. Our proposed mechanism (Scheme I) requires that each of these inhibitors form the same inhibitor-enzyme complex, and that reactivated HLE would form by the same mechanism (through the same rate determining step). The inactivation rates (k_{inact}) and apparent binding constants (K_i^*) vary 200 fold (190 to

44,000 $M^{-1}sec^{-1}$ for k_{inact}), but the reactivation rates (k_{react}) vary less than two fold (4.7×10^{-5} to 8.4×10^{-5} sec^{-1}). The reactivation rates are very similar which suggests that reactivation of HLE occurs through the same intermediate which we propose is a cross linked inhibitor-enzyme complex (see Scheme I). The rates of HLE inactivation by these inhibitors would be largely dependent on enzyme binding interactions and on the leaving group ability.

Table 2. 4-Position Substituent Effects on HLE Inhibition.



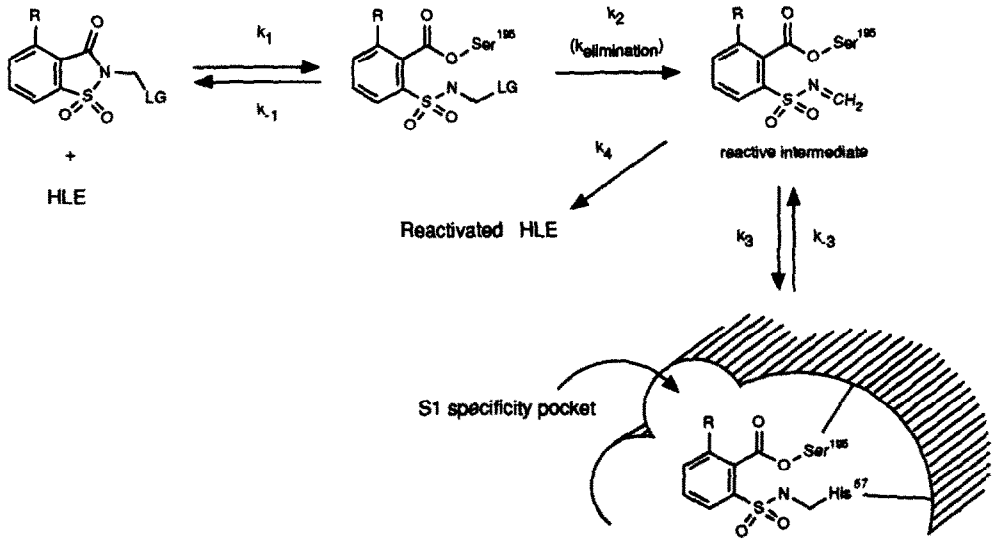
Cmpd	R =	HLE k_{inact} ($M^{-1}sec^{-1}$)	HLE k_{react} (sec^{-1})	HLE K_i^* (nM)
7	H	5,600	0.000084	15
9	CH_3	3,200	0.00010	32
10	CH_2CH_3	63,200	0.00013	2
11	$CH_2CH_2CH_3$	100,000	0.000070	0.7
12	$CH(CH_3)_2$	94,000	0.000028	0.3
13	$CH(CH_3)(CH_2CH_3)$	94,000	0.000056	0.6
14	$CH(CH_2CH_3)_2$	4,400	0.00026	60
15	$C(CH_3)_3$			>2000
16	C_6H_5	5,800	0.00046	80
17	Br	24,000	0.00036	15
18	OCH_3	5,600	0.000076	13.6
19	OCH_2CH_3	45,300	0.00014	3
20	$OCH(CH_3)_2$	8,900	0.00016	17.5
L-658,758		3,700	0.0000081	2.2
ICI-200,355		94,000	0.000037	0.4

Computer modeling by docking substituted benzisothiazolones predicted that substituents at the 4-position would interact at the S1 specificity pocket of HLE. Specifically, small lipophilic groups would exert a positive hydrophobic interaction with the S1 pocket and lead to compounds with more rapid inactivation rates and thereby improved inhibitor potency.[13] The 4-alkyl analogues (**9-16**) were prepared for testing,[14] and were shown to lead to an increase of the inactivation rate and inhibitor potency, presumably due to a favorable lipophilic interaction with the S1 pocket. Comparison of the 4-hydrogen to the 4-isopropyl analogue shows a 17 fold increase in the inactivation rate and a modest 3 fold decrease in the reactivation rate, however, the combined effect of these two rates gave *a 50 fold increase* in the apparent binding constant. The 4-isopropyl compound **12** was the most potent inhibitor in the series.

The stoichiometry of inhibition by the achiral **10** was 1.1:1.0, indicating that one molecule of compound **10** (WIN 62225) inactivates one molecule of HLE. The stoichiometry of HLE inhibition by **13** was 1.9:1.0 suggesting that one enantiomer of **13** was significantly more potent than the other. Since the S1 pocket of HLE is chiral, one enantiomer of the s-butyl compound **13** should readily fit into the pocket, while the other enantiomer should not. To test this idea, the 4-(3-pentyl) derivative **14** was prepared, and was significantly less potent than the 4-isopropyl or 4-(s-butyl) analogues. This result is consistent with the modeling hypothesis, since overlapping of the two enantiomers of s-butyl gives a 3-pentyl group. The computer model showed significant non-bonded interactions of the 3-pentyl group with the surface of HLE in the model.

An electron withdrawing substituent in the 4-position increased the inactivation rate relative to the 4-H compound **7**. However, compound **17** (4-Br) is equipotent to **7**, because the reactivation rate increased proportionately. Electron donating 4-alkoxy substituents (**18-20**) did not lead to more potent inhibitors. A mechanism-based inhibitor (L-658,758)[3] and a transition state mimetic (ICI-200,355)[18] are given as reference compounds in Table 2.

Scheme I. Proposed Mechanism of Inhibition.



We have discovered a new class of potent mechanism-based inhibitors of human leukocyte elastase. The 4-isopropyl analogue, WIN 62785 (**12**), is the most potent compound in this new class of HLE inhibitors with a K_i^* = 0.3 μM. The 4-ethyl analogue, WIN 62225 (**10**), is a selective, stoichiometric HLE inhibitor (K_i^* = 2.0 nM). WIN 62225 inhibits porcine pancreatic elastase (K_i^*=20 nM). Selectivity was noted for HLE over chymotrypsin (K_i^*=90 nM), but specificity was obtained over the trypsin-like human enzymes thrombin and plasmin (K_i^*>60,000 nM).

General synthetic method for the preparation of compounds 3-20:

4-(1-Methylethyl)-2-[(phenylthio)methyl]-1,2-benzisothiazol-3(2H)-one 1,1-dioxide (I). A mixture of 4-(1-methylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide (37.9 g, 0.168 mol),[15] chloromethyl phenyl sulfide (33.3 g, 0.21 mol), tetrabutylammonium bromide (5.4 g, 17 mmol), and 200 mL of toluene was stirred and refluxed for 24 h. Heating was stopped and the mixture was evaporated under reduced pressure. The residue was taken up 1:1 CH_2Cl_2:hexane and added to a column of 485 g of silica gel. The column was eluted with hexane and then 1:1 CH_2Cl_2:hexane. Evaporation of the 1:1 CH_2Cl_2:hexane fractions gave 53.5 g (92%) of **I** as a yellow oil: NMR ($CDCl_3$) δ 1.25 (d, J = 6 Hz, 6H), 4.21 (m, 1H), 5.13 (s, 2H), 7.32 (m, 3H), 7.61 (m, 2H), 7.75 (m, 3H); MS 348 (MH^+). **2-(Chloromethyl)-4-(1-methylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide (II).** The benzisothiazol **I** (53.5 g, 0.154 mol), sulfuryl chloride (67.2 g, 0.50 mol) and 250 mL of CH_2Cl_2 were stirred together for 1 hr and allowed to stand overnight. The solvent was removed under reduced pressure and the residue was taken up in 100 mL of hexane and scratched. Solid separated and was collected after cooling to afford 38.55 g of cream solid. Recrystallization from isopropanol-cyclohexane gave 33.5 g (80%) of **II** as a colorless solid: mp 101-102.5 °C; NMR ($CDCl_3$) δ 1.33 (d, J = 6 Hz, 6H), 4.30 (m, 1H), 5.56 (s, 2H), 7.81 (m, 3H); MS 274 (MH^+), 238 (M^+-35). Anal. Calcd for $C_{11}H_{12}ClNO_3S$: C, 48.27; H, 4.42; N, 5.12. Found: C, 48.37; H, 4.55; N, 5.14. **4-(1-Methylethyl)-2-[(1-phenyl-tetrazol-5-ylthio)methyl]-1,2-benzisothiazol-3(2H)-one 1,1-dioxide (12).** A mixture of the benzisothiazol **II** (0.20 g, 0.73 mmol), 1-phenyl-5-mercaptotetrazole sodium salt (0.16 g , 0.80 mmol), and 4 mL of dimethylformamide was stirred at 110°C for 1 hr. On cooling the mixture was poured onto saturated sodium bicarbonate and water, then extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered, concentrated under reduced pressure, and the residue was purified by flash chromatography (SiO_2, 1:4 ethyl acetate:hexanes). A colorless solid (0.23 g) was obtained and crystallized from ethanol to give 0.17 g (56%) of **12** as colorless crystals: mp 140-141°C; IR 1726 cm^{-1}; NMR ($CDCl_3$) δ 1.22 (d, J = 6.89 Hz, 6H), 4.04 (m, 1H), 5.55 (s, 2H), 7.58 (s, 5H), 7.93 - 8.11 (m, 3H); MS 416 (MH^+). Anal. Calcd for $C_{18}H_{17}N_5O_3S_2$: C, 52.04; H, 4.12; N, 16.86. Found: C, 51.84; H, 3.99: N, 16.77.

HLE Kinetic Assay. Inhibition kinetics for compounds were determined by a spectrophotometric assay that follows the release of p-nitroaniline from the substrate MeOSuc-Ala-Ala-Pro-Val-pNA at 410 nM (concentration = 200 μM, K_m = 50 μM). Elastase (5 nM) was added to a mixture of substrate and inhibitor at room temperature to initiate the reaction. Inhibitor concentrations ranged from 50-25000 nM. All assays contained 7.5% DMSO. Most inhibitors exhibited time-dependent ("slow, tight-binding") inhibition; assays were run over the course of 5 hours to ensure that a steady-state rate had been reached. Progress curves from the assays containing inhibitor were fit by non-linear least square methods to an integrated rate equation to yield final steady-state velocity (v_f) and the pseudo-first-order rate constant for inactivation (k_{obs}). Control assays which did not contain inhibitor were fit by linear regression to yield control velocities (v_o). The K_i^* for inhibitors were determined by regression of a plot of $[I]/(1-v_f/v_o)$ versus v_o/v_f, K_i^* = slope/(1 + [S]/K_m). The k_{inact}'s were determined by linear regression of a plot of k_{obs} versus [I]; k_{inact} = slope * (1 + [S]/K_m). The reactivation rate of inhibitors (k_{react}) were calculated from $K_i^* = k_{react}/k_{inact}$.

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13. During the course of this work, based on SAR results Krantz (ref. 6) has suggested that the improved potency of 5-alkyl benzoxazinones as alternate-substrate inhibitors may be due to an interaction with the S1 pocket. This position is analogous to the 4-position of benzisothiazolones.
14. The starting 1,2-benzisothiazol-3(2H)-one 1,1-dioxides were prepared by published methods (see ref. 15-17). The introduction of the *N*-methyl leaving groups is described in the experimental section, along with the HLE kinetic assay.
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