JOURNAL OF
MEDICINAL
CHEMISTRY

Volume 37, Number 17 August 19, 1994

Communications to the Editor

2,6-Disubstituted Aryl Carboxylic Acids, Leaving Groups "Par Excellence" for Benzisothiazolone Inhibitors of Human Leukocyte Elastase

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Received June 21, 1994

The pathological involvement of human leukocyte elastase (HLE) in the mediation of various pulmonary disorders like emphysema,[1] acute respiratory distress syndrome,[2] cystic fibrosis,[3] and chronic bronchitis[4] has attracted considerable interest in the synthesis of small molecular weight inhibitors of this serine proteinase. Such inhibitors could be of use in the treatment of these disease states.[5] Recently, Hlasta et al. reported the discovery of potent and selective benzisothiazolone based inhibitors (**1**) of HLE.[6] It was clearly demonstrated that the activity in this series was dependent on two important factors, namely the R_4 substituent and the nature of the leaving group positioned on the nitrogen of the benzisothiazolone nucleus. Herein, we would like to report that various aryl carboxylates were found to be very effective leaving groups, and in conjunction with the optimum R_4 substituent led to very potent and in vivo active inhibitors of HLE.[7]

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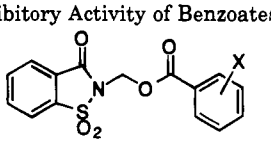
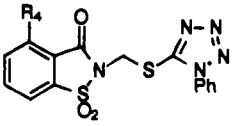
Table 1. HLE Inhibitory Activity of Benzoates **2a–h**

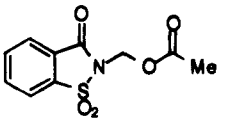


compd	X	elastase inhibition[a] pK_a of acid[b]	k_{inact} (M^{-1} s^{-1})	K_i* (nM)
1b	–	4.76	630	102
2a	H	4.20	3400	31
2b	2,6-Cl_2	1.59	24500	2.7
2c	2,6-Br_2	1.5[c]	9300	8.5
2d	2,6-F_2	2.24	10000	8
2e	2,6-$(OMe)_2$	3.44	6300	8.0
2f	2-Cl, 6-NHAc	–	3700	18
2g	2,6-Me_2	3.35	16000	4
2h	2,6-$(CF_3)_2$	0.58	1500	37

[a] See ref 6 for enzyme kinetic method. [b] The pK_a values are from ref 9 except as noted. [c] Calculated pK_a according to the method given in ref 10.



1a, R_4 = H, Alkyl or Aryl



1b

Our interest in exploring the use of aryl carboxylates as leaving groups stems from a report by Krantz and co-workers that they are effective nucleofuges in the design of peptidyl (acyloxy)methyl ketone inhibitors of a number of cysteine proteases.[8] However, a similar exercise by Krantz in the design of peptidyl (acyloxy)-methyl ketone inhibitors of HLE has been reported to be unsuccessful.[9] They rationalized this result by invoking that the OH group of Ser^{195} in serine proteases lack the nucleophilicity necessary for an S_N2 like displacement of the carboxylate leaving group in a peptidyl (acyloxy)methyl ketone. Since the proposed mechanism of inhibition of HLE (Figure 1)[6,7] by the benzisothiazolones does not involve direct displacement of the leaving group (by Ser^{195}), we postulated that aryl carboxylates would be effective nucleofuges in improving the potency of this class of inhibitors. Moreover, the aryl carboxylate leaving groups provide an opportunity

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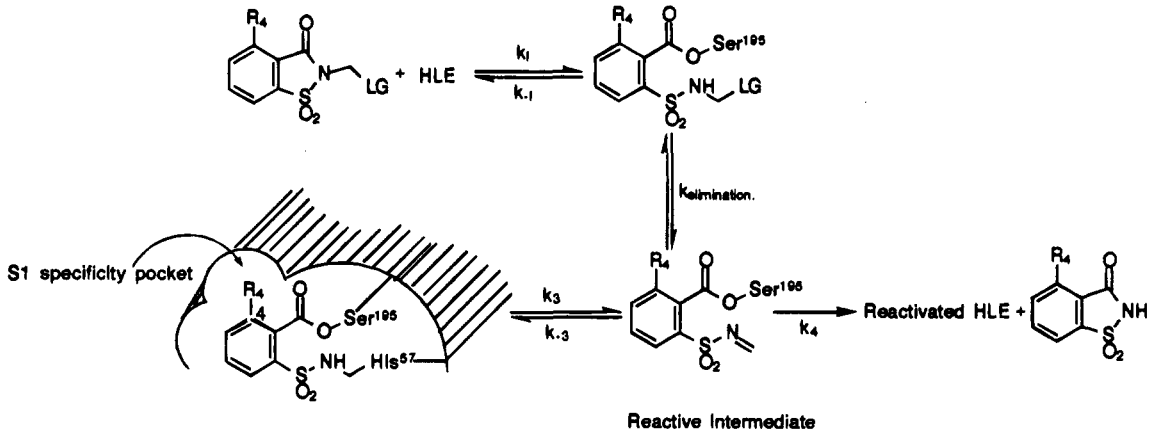
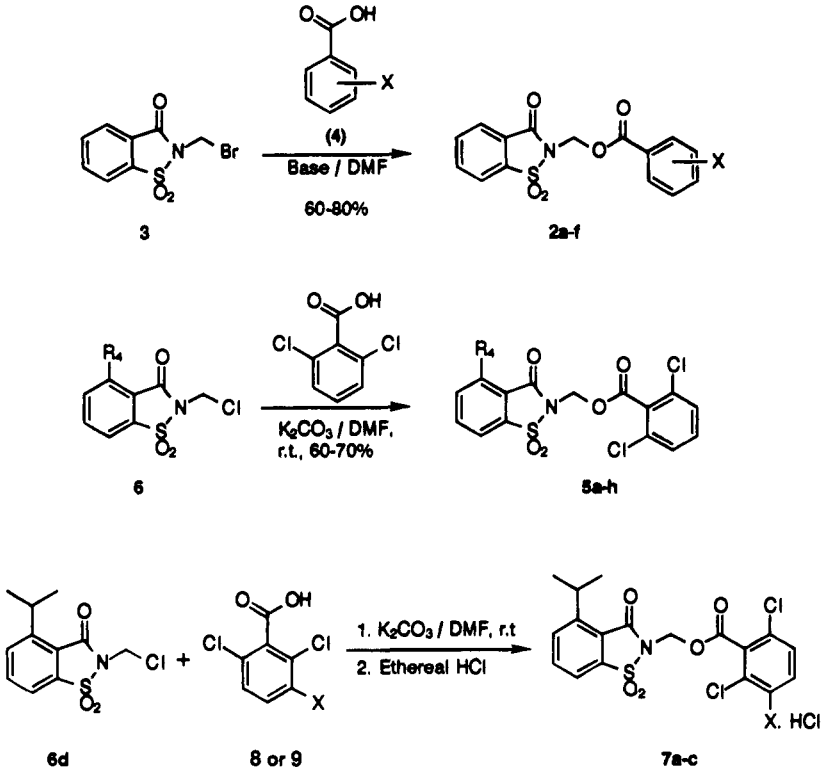


Figure 1. Proposed mechanism for the inhibition of HLE by benzisothiazolone based inhibitors.

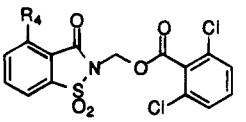
Scheme 1



for structural variations that could lead to desired pharmacokinetic properties and aqueous solubility, which we found necessary for in vivo activity (vide infra).

In order to prevent undesirable nucleophilic attack on the benzoyloxycarbonyl group of our target structures **2**, we chose sterically hindered 2,6-disubstituted benzoates as leaving groups. The desired aryl carboxylates **2a–h** were prepared (Scheme 1) by reaction of readily available bromide **3**[10] with the appropriate carboxylic acids **4** in the presence of a base (K_2CO_3 or TlOEt or Et_3N). The HLE inhibitory activity of compounds **2a–h** is shown in Table 1. Of the various substituents explored, compound **2b** having a 2,6-dichlorobenzoate leaving group was the most potent. Replacement of chlorine with other halogens (compounds **2c** and **2d**) led to a 4-fold decrease in potency. Introduction of electron-donating groups at either the 2 or 6 or both positions of the benzoic acid (compounds **2e** and **2f**) also led to a drop in HLE inhibitory activity. The dimethyl analog **2g** was only slightly less potent than the dichloro compound **2b**. On the contrary, the bis(trifluoromethyl) compound **2h** was 10-fold less potent than the cor-

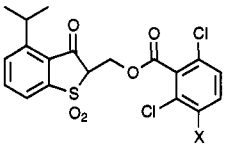
Table 2. Effect of C-4 Substitution on HLE Activity of 2,6-Dichlorobenzoates **5a–f**

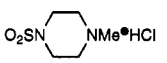
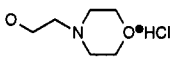


compd	R_4	HLE inhibitory activity k_{inact} (M^{-1} s^{-1})	HLE inhibitory activity K_i* (nM)
2b	H	24500	2.7 (15)[a]
5a	Me	38000	2.0 (32)
5b	Et	700000	0.17 (2)
5c	*i*-Pr	900000	0.03 (0.3)
5d	*s*-Bu	940000	0.06 (0.07)
5e	*t*-Bu	6200	26 (>2000)
5f	Ph	460000	1.0 (80)

[a] The value in parentheses are for the corresponding compounds with a 1-phenyl-5-mercaptotetrazole leaving group. Data is taken from ref 6.

Table 3. In Vitro and in Vivo inactivation of HLE by 2,6-Dichlorobenzoates **7a–c**



compd	X	in vitro HLE inhibition		in vivo HLE activity[a]		
		k_{inact} (M^{-1} s^{-1})	K_i* (nM)	% inh. at 1 mg/kg.	% inh. at 10 mg/kg	ED_{50} (mg/kg)
5c	H	900000	0.03	–	47%[b]	ND[c]
7a		3000000	0.007	57 ± 12	89 ± 3	1
7b	$SO_2N(Me)CH_2CH_2NMe_2$·HCl	2300000	0.01	95 ± 3	97 ± 6	0.6
7c		2875000	0.008	87 ± 6	99 ± 5	ND
MeOSucAAPVCMK	–	3150	–	ND	ND	0.08

[a] The data reported is for the test compound administered intravenously 1 min prior to the intratracheal administration of HLE (25 μg) in hamsters. See ref 14 for a detailed description of the elastase induced pulmonary hemorrhage model in hamsters. [b] The compound could not be administered intravenously because of its extreme insolubility in aqeous medium. The percent inhibition is for drug administered subcutaneously at 60 mg/kg and a pretreatment time of 60 min before HLE was administered. [c] ND = not determined.

responding dichloro analog **2b**, which is opposite to that observed by Krantz with their aryl (acyloxy)methyl ketone inhibitors of cathepsin B.[8] The reason for the loss of potency observed with compound **2h** is not very obvious. Although the data suggests that a threshold pK_a value of <4 for the leaving group carboxylic acid (Table 1) is needed for effective inhibition, this is not the only factor that influences HLE inhibitory activity. The decrease in potency with compound **2h** (wherein the corresponding carboxylic acid has the lowest pK_a value among those used) and the small difference in activity between compounds **2f** and **2g** (even though the pK_a of the acids differ by 2 units) clearly indicate that there are other factors (such as interaction of substituents on the leaving group with the surface of the protein) that influence the activity of this class of HLE inhibitors.

Since placement of small hydrophobic alkyl groups at C-4 has been shown[6] to increase potency in the benzisothiazolone class of HLE inhibitors, the C-4 substituted compounds **5a–f** with a 2,6-dichlorobenzoate leaving group were prepared. The synthesis of these targets were achieved from the known benzisothiazolones **6**[6] by alkylation with dichlorobenzoic acid in the presence of K_2CO_3 in DMF at room temperature.

The SAR for the C-4 substituent for inhibitors **5a–f** was similar to that reported[6] for the corresponding compounds with a 1-phenyl-5-mercaptotetrazole leaving group with potency in the 2,6-dichlorobenzoate series being 10–80-fold better (Table 2). The improvement in activity with the benzoates is solely due to a 10-fold increase in inactivation rates. Compound **5c** with a 4-isopropyl substituent was the most potent among this series with a $k_{inact} = 9 \times 10^5$ M^{-1} s^{-1} and a K_i* = 0.03 nM. Despite its excellent in vitro potency, compound **5c** showed very little activity in an elastase-induced pulmonary hemorrhage model in hamsters.[12] A liability for this compound was its extremely poor aqueous solubility, preventing its effective administration by either an iv or sc route. Even during instances where it could be administered subcutaneously, the observed in vivo activity was poor. To overcome this problem and impart aqueous solubility to **5c**, we introduced polar substituents at the C-3′ position of the aryl carboxylate phenyl ring, because molecular modeling studies[13,14] performed by docking compound **5c** into the active site of HLE indicated that this region of the molecule is pointed toward solvent, and the initial enzyme–inhibitor complex should tolerate such hydrophilic substituents.

The desired benzoates **7a–c** were prepared by alkylation of the readily available benzoic acids **8** or **9**[7a] with the chloromethyl compound **6c**. As seen in Table 3, introduction of these aqueous solubilizing substituents not only led to the desired in vivo activity but also resulted in a 3–4-fold increase in the in vitro inhibitory potency against HLE. The suicide inhibitor MeOSuc-Ala-Ala-Pro-Val-CH_2Cl (MeOSucAAPVCMK)[16] is shown as a reference compound in Table 3.

In summary, we have discovered very potent and in vivo active non-peptide inhibitors of human leukocyte elastase based on the benzisothiazolone nucleus with a 2,6-dichlorobenzoate leaving group. Compound **7a** (WIN 63110) with a K_i* = 0.007 nM is the most potent ($k_{inact} = 3 \times 10^6$ M^{-1} s^{-1})[17] small molecule inhibitor reported for a serine protease and **7a** inhibits HLE in vivo with an ED_{50} = 1 mg/kg (iv). The k_{inact} for this compound is only 10-fold less than that reported[18] for α_1-PI ($k_{inact} = 6.5 \times 10^7$ M^{-1} s^{-1}), a naturally occurring and essentially irreversible inhibitor of HLE. Work is underway to understand why the aryl carboxylates lead to such a dramatic improvement in inactivation rates. Results of these investigations and our efforts to structurally modify this class of HLE inhibitors to achieve oral bioavailability will be the subject of future publications.

Acknowledgment. We thank Drs. Ranjit Desai, Arup Ghose, and James Kofron for helpful discussions and Mr. Al Hlavac and George Gonyea of the department of physical chemistry and molecular characterization for high-resolution mass spectra.

Supplementary Material Available: General synthetic procedures for all compounds (2 pages). Ordering information is given on any current masthead page.

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(13) Enzyme–ligand modeling studies were performed using BIOGRAF and the geometry optimisations were done using Dreiding Force Field in BIOGRAF.

(14) Compound **5c** was docked into the active site of HLE in such a manner that the *i*-Pr group is bound to the S1 specificity pocket of HLE (see ref 15 for a detailed description of the S1 specificity pocket of HLE) and the carbonyl of the benzisothiazolone moiety is constrained into the oxyanion hole by co-valently linking it with Ser^{195}. A complete description of the modeling studies will be reported in a full account of this work.

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