Notes

Phosphonates and Phosphinates: Novel Leaving Groups for Benzisothiazolone Inhibitors of Human Leukocyte Elastase†

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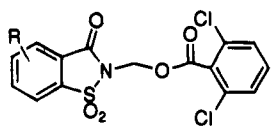
Received December 24, 1994®

A novel class of alkyl and aryl phosphonate and phosphinate acid-based leaving groups has been developed for utilization in the synthesis of benzoisothiazolone (BIT) inhibitors of human leukocyte elastase (HLE). A number of BITs were synthesized with phosphonate and phosphinate acid-based leaving groups and were found to be potent inhibitors of HLE. Compound **3c** with a diethyl phosphonate leaving group is the most potent inhibitor synthesized in this series with $K_i^* = 0.035$ nM and $ED_{50} = 2.0$ mg/kg.

The degranulation of neutrophils in response to inflammatory stimuli results in the release of several defensive serine proteases including human leukocyte elastase (HLE).[1] Uncontrolled free HLE is capable of proteolytic degradation of a wide range of proteins, e.g., elastin, collagen, fibrinogen, etc. Excessive elastolytic activity has been postulated in the pathology of several respiratory disorders such as pulmonary emphysema,[2] cystic fibrosis,[3] and adult respiratory distress syndrome (ARDS).[4] Administration of low molecular weight HLE inhibitors should prove to be a therapeutically useful strategy against the aforementioned pathologies.

We have recently reported the discovery of potent, mechanism-based benzisothiazolone (BIT) inhibitors of HLE, **1**.[5] The proposed mechanism of inhibition of HLE



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by the BIT analogs is shown in Figure 1. We have reported that the HLE inhibitory activity of the BIT-based analogs can be effectively modulated by two distinct factors: the R_4 and R_6 substituents on the BIT nucleus and the nature of the leaving group.[5] In order to broaden our search for useful leaving groups, we initiated a study to explore a variety of functionalities other than mercaptotetrazoles,[5c] aryl carboxylates,[5b] and phenols[6] to serve as leaving groups. One distinctly novel class of leaving group that has emerged from this study is based on the phosphorous acids. To the best of our knowledge, the use of this class of compounds as leaving groups in the synthesis of serine proteinases inhibitors has not been described previously.[7,8] Herein, we report that various alkyl and aryl phosphonates and phosphinates were found to be very efficacious leaving groups and in conjunction with the optimum R_4 and R_6 substituents led to highly potent and *in vivo* active HLE inhibitors.

Results and Discussion

The desired target compounds **2a–g** and **3a–c** were synthesized as shown in Scheme 1. The synthesis of compound **3d** is shown in Scheme 2. The coupling of the known intermediate bromide **4**[5b] and chloride **5**[9] with the appropriate phosphorous acids was carried out in the presence of base (Et_3N or *N,N*-diisopropylethylamine). The HLE inhibitory activity of compounds **2a–g** is described in Table 1. As seen from the data in Table 1, there is a gradual increase in the HLE inhibitory activity in going from the dimethyl phosphonate analog **2a** to the corresponding dibutyl phosphonate analog **2c**. Compound **2d**, with a diphenyl phosphonate leaving group, was found to be the most potent. With the diphenyl phosphinate leaving group in **2f**, the potency dropped 4.6-fold. The similar trend was also observed for compounds **2c** and **2g**, where a 1.4-fold difference in potency was observed between the dibutyl phosphonate and the corresponding dibutyl phosphinate leaving group. This difference in potency between the phosphonate- and phosphinate-based leaving groups may be rationalized on the basis of the difference in the pK_a values of the corresponding phosphorous acids. The phosphonates have comparatively lower pK_a values than the corresponding phosphinates and are superior leaving groups (Table 1). Although the difference in the potency between these two sets of inhibitors may be explained based on the pK_a values, the data in Table 1 suggests that there is not a direct correlation between the pK_a values and the

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® Abstract published in *Advance ACS Abstracts*, April 1, 1995.

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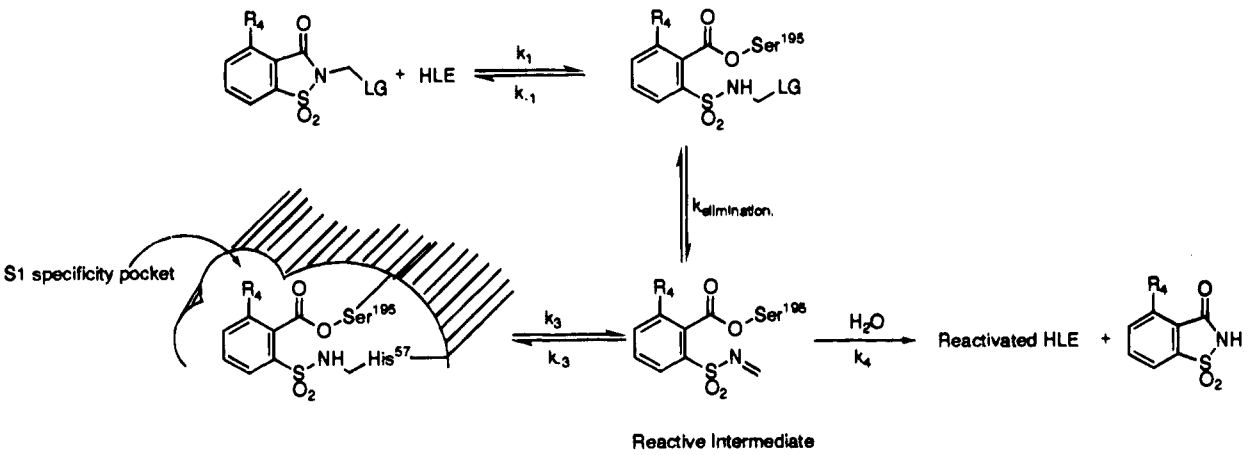
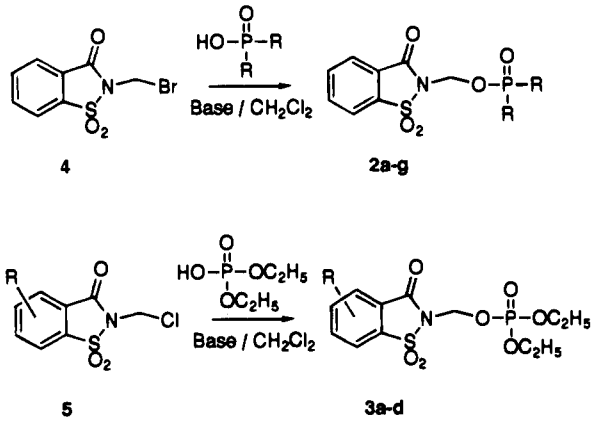
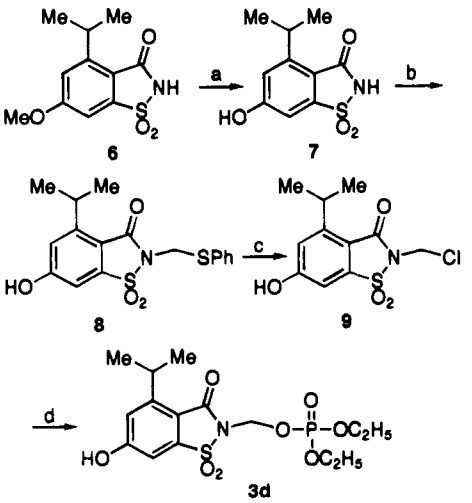


Figure 1. Proposed mechanism for the inhibition of HLE.

Scheme 1



Scheme 2[a]

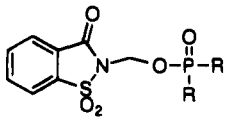


[a] Reagents: (a) $AlCl_3$, EtSH, $CHCl_3$; (b) $C_6H_5SCH_2Cl$, iPr_2NEt, toluene; (c) SO_2Cl_2, CH_2Cl_2; (d) diethyl phosphate cesium salt, DMF.

HLE inhibitory activity. Other factors, such as the interaction of the leaving group with the surface of the protein, contribute to the potency of these inhibitors.

To select the best leaving group for further analog synthesis, we evaluated compounds **2b–g** in the hamster HLE-induced hemorrhage model.[11] Unfortunately, with the exception of **2b**, other analogs could not be evaluated in the hamster model because of poor aqueous solubility. Compound **2b** was administered by the subcutaneous route and showed 56% inhibition at a dose

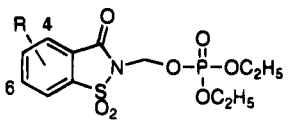
Table 1. HLE Inhibitory Activity of Compounds **2a–g**



compd	R	pK_a of acid[b]	HLE inhibitory activity[a] k_{inact} (M^{-1} s^{-1})	HLE inhibitory activity[a] K_i* (nM)
2a	OCH_3	2.56	800	100
2b	OC_2H_5	2.51	1100	60
2c	$O(CH_2)_3CH_3$	2.59	4800	17
2d	OC_6H_5	1.91	11000	6.5
2e	$OCH_2C_6H_5$	2.26	4100	20.5
2f	C_6H_5	3.34	1900	30
2g	$(CH_2)_3CH_3$	4.62	2300	24

[a] See ref 5c for enzyme kinetic method. [b] Calculated pK_a according to the method given in ref 12.

Table 2. *In Vitro* and *In Vivo* Inactivation of HLE by Diethyl Phosphonates **3a–d**



compd	R	*in vitro* HLE inhibition k_{inact} (M^{-1} s^{-1})	*in vitro* HLE inhibition k_{react} (s^{-1})	*in vitro* HLE inhibition K_i* (nM)	*in vivo* HLE activity[a] % inhibn at 10 mg/kg
2b	H	1 100	0.000 066	60	56 ± 8 (b)
3a	R_4 = *i*-Pr	530 000	0.000 053	0.1	84 ± 3 (c)
3b	R_4 = *s*-Bu	800 000	0.000 056	0.07	83 ± 4 (b)
3c	R_4 = *i*-Pr, R_6 = OMe	343 000	0.000 012	0.035	92 ± 3 (c)
3d	R_4 = *i*-Pr, R_6 = OH	3 300	0.000 014	4.5	

[a] The data reported is for the test compound administered subcutaneously (b) or intravenously (c) 1 min prior to the intratracheal administration of HLE (25 μg) in hamsters. See ref 12 for a detailed description of the elastase induced pulmonary hemmorhage model in hamsters.

of 10 mg/kg. Encouraged by this result, we pursued the synthesis of various substituted BIT analogs with the diethyl phosphonate leaving group (Table 2).

As reported previously,[5c] small lipophilic substituents at the 4-position of the BIT nucleus exert a positive hydrophobic interaction with the S_1 specificity pocket of the enzyme and led to compounds with rapid inactivation rates. Substitution of 4-H with an isopropyl group as in compound **3a** increased the inactivation rate *481-fold* in comparison with that of **2b** and increased

potency *600-fold* (Table 2). The 4-*sec*-butyl substituent in the analog **3b** increased the inactivation rate *727-fold*, reflecting a superior potency when compared to **3a**. Replacement of 6-H with an electron-donating methoxy group resulted (compare **3c** with **3a**) in a *1.5-fold* decrease in the inactivation rate, reflecting the decrease in the electrophilicity of the BIT carbonyl group; however, the corresponding reactivation rate also decreased 4.4-fold. The net outcome of these two rate differences resulted in a 2.8-fold improvement in potency for **3c**. As pointed out in the previous publication,[5a] the superior potency of compound **3c** may be in part due to a hydrogen bond formation between the C-6 methoxy group and the valine^{216} NH of the elastase. Replacing the 6-methoxy substituent with 6-hydroxy group as in analog **3d** resulted in *128-fold* decrease in potency in comparison to that of **3c**. This result is not surprising since at pH 7.8 (*in vitro* assay pH), the phenol of **3d** is expected to ionize and the resulting phenoxide would considerably reduce the reactivity of the BIT carbonyl group toward the serine hydroxyl of the elastase. The three compounds **3a–c** showed excellent *in vivo* activity in the hamster model (Table 2). Compound **3c** with an ED_{50} of 2 mg/kg is the most potent analog synthesized in this series.

In summary, we have discovered a novel class of phosphorous acid-based leaving groups.[13] The unprecedented utility of the alkyl and aryl phosphonate and phosphinate leaving groups in the design of serine proteinase inhibitors has been demonstrated with the synthesis of highly potent and *in vivo* active inhibitors of human leukocyte elastase.

Experimental Section

Unless otherwise noted, materials were obtained from commercial sources and used without further purification. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet 20SX FTIR. NMR spectra were acquired in the indicated solvent on a JEOL-FX270, General Electric QE-300, or Bruker AC200 FT NMR, and the chemical shifts are expressed in δ units and were referenced to chloroform at δ 7.26, and signal multiplicity was designated according to the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, sep = septet. Mass spectra were recorded on a Nermag R 10/10 coupled to a Varian 3400 gas chromatograph or on a JEOL-01SC spectrometer. High-resolution mass spectra were recorded using liquid secondary ionization mass spectrometry (LSIMS) on a high-resolution double focusing instrument, Kratos' Concept. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, or by QTI technologies.

General Method for the Preparation of Phosphinates 2f,g and Phosphonates 2a–e and 3a–c. The procedure described below for the preparation of diethyl phosphonate **3c** is typical. To a solution of 2-(chloromethyl)-4-isopropyl-6-methoxy-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide (1.0 g, 3.3 mmol) in CH_2Cl_2 (25 mL) containing Et_3N (0.7 mL, 4.45 mmol) was added diethyl phosphate (0.76 g, 4.93 mmol), the mixture was heated to reflux for 72 h, cooled, and concentrated under reduced pressure, and the residue obtained was subjected to flash chromatography (SiO_2, 1:1 EtOAc–hexane) to furnish compound **3c** (0.92 g, 66% yield) as an oil: IR (film, cm^{-1}) 1735, 1607, 1565, 1340, 1028, 1005; ^1H NMR ($CDCl_3$) δ 1.22–1.39 (m, 12H), 3.97 (s, 3H), 4.19 (sep, 1H, J = 7.3 Hz), 5.67 (d, 2H, J = 7.67 Hz), 7.19–7.23 (m, 2H). Anal. ($C_{16}H_{24}NO_8PS$) C, H, N.

Phosphonate 3d. The starting 2-(chloromethyl)-4-isopropyl-6-hydroxy-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide (**9**) used in the preparation of compound **3d** was synthesized from 4-isopropyl-6-methoxybenzisothiazolone (**6**). Thus, to a suspension of $AlCl_3$ (62.74 g, 0.47 mol) in dry $CHCl_3$ (500 mL) at 0 °C was added ethanethiol (52.2 mL, 0.7 mol).[14] Within minutes, the suspension turned into a clear solution. To this solution was then added under cooling (0 °C) a solution of **6** (20 g, 0.078 mol) in $CHCl_3$ (550 mL) over a period of 30 min. After the addition was over, the solution was allowed to warm to room temperature, then heated at 60–65 °C (bath) for 3–4 h, cooled, poured over ice–water, and acidified with dilute HCl. The precipitate was filtered and washed with water and dried to give **7** as a white solid (18.4 g, 97%): mp 249.5–251.5 °C; ^1H NMR (DMSO-d_6) δ 1.11 (d, 6H, J = 6.96 Hz), 3.98 (sep, 1H, J = 6.9 Hz), 6.94 (s, 2H).

To a suspension of **7** (50.0 g, 0.21 mol) in toluene (500 mL) containing DMF (50 mL) was added diisopropylethylamine (37.8 mL, 0.22 mol) and chloromethyl phenyl sulfide (29.16 mL, 0.22 mol). The suspension was heated to reflux for 24 h, cooled, washed with water, 5% aqueous HCl, and brine and dried. The filtrate was concentrated, and the residue was purified on a flash column using 10–30% EtOAc–hexane to furnish 58 g (77% yield) of **8**: mp 150–151.15 °C; ^1H NMR ($CDCl_3$) δ 1.21 (d, 6H, J = 6.9 Hz), 4.07 (sep, 1H, J = 6.9 Hz), 5.1 (s, 2H), 6.61 (s, 2H), 7.1–7.6 (m, 7H). Anal. ($C_{17}H_{17}NO_4S$) C, H, N.

Sulfuryl chloride (6.08 mL, 0.076 mol) was added at room temperature to a solution of **8** (25 g, 0.069 mol) in CH_2Cl_2 (250 mL), the mixture was stirred for 3–4 h and concentrated, and the residue was suspended in hexane and stirred for 1 h during which the oily residue solidified, and was filtered, washed with hexane, and dried to provide 17.23 g (86% yield) of **9**: mp 149–150 °C; ^1H NMR ($CDCl_3$) δ 1.28 (d, 6H, J = 7.0 Hz), 4.17 (sep, 1H, J = 6.9 Hz), 5.54 (s, 2H), 7.15 (d, 1H, J = 2.0 Hz), 7.18 (d, 1H, J = 2.0 Hz).

The cesium salt of diethyl phosphate was made by treating diethyl phosphate (0.42 g, 2.73 mmol) in MeOH (10 mL) with cesium carbonate (0.44 g, 1.35 mmol) at room temperature for 2 h (N_2), the mixture was concentrated, and the residue obtained was dried under high vacuum. This salt was suspended in DMF (10 mL), to this was added compound **9** (0.4 g, 1.38 mmol), and the mixture was stirred at 50 °C for 15 h, cooled, poured over ice–water, extracted with EtOAc (2 × 50 mL), washed with water and brine, and dried (Na_2SO_4). Solvent removal under reduced pressure followed by purification using flash chromatography (SiO_2, 6:4 EtOAc–hexane) provided the desired compound **3d** (0.3 g, 53%): mp 127.5–128.5 °C; IR (KBr, cm^{-1}) 3041, 2978, 1734, 1620, 1569, 1086; ^1H NMR ($CDCl_3$) δ 1.23 (d, 6H, J = 6.9 Hz), 1.37 (t, 6H, J = 6.1 Hz), 4.05–4.29 (m, 5H), 5.67 (d, 2H, J = 10.4 Hz), 7.09 (d, 1H, J = 2.1 Hz), 7.35 (d, 1H, J = 2.1 Hz). Anal. ($C_{15}H_{22}NO_8PS$) C, H, N.

Acknowledgment. We thank Dr. Joost Strasters for the pK_a measurements and Mr. Al Hlavac for recording high-resolution mass spectra.

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JM940834Q