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ALKOXY SUBSTITUTED BENZISOTHIAZOLONE (BIT) DERIVATIVES: POTENT INHIBITORS OF HUMAN LEUKOCYTE ELASTASE.

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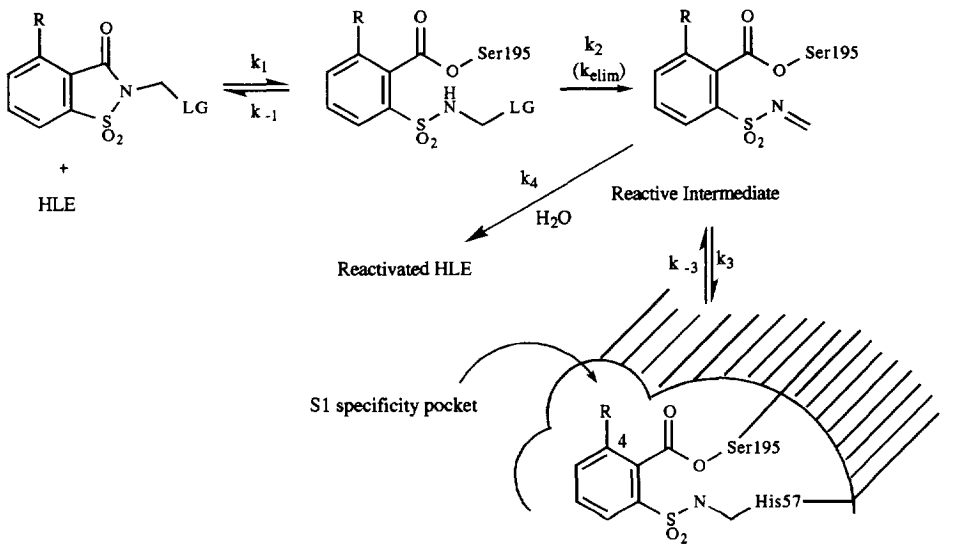
Abstract. Alkoxy substituted benzisothiazolones (BIT) are reported as inhibitors of human leukocyte elastase (HLE). Structure-activity relationship study results are described. The contribution of alkoxy substituents towards improving the stability of BIT derivatives in human blood is discussed. WIN 68769 (**12**) with a K_i^* = 0.022 nM is the most potent analog synthesized in this series.

Inhibitors of the serine proteinases, in particular human leukocyte elastase (HLE), have attracted considerable attention. There has been an intensive effort to develop low molecular weight inhibitors of HLE,[1] an enzyme implicated in the etiology of several pulmonary disease states such as emphysema,[2] acute respiratory distress syndrome,[3] cystic fibrosis,[4] and chronic bronchitis.[5] We have recently reported a novel class of potent mechanism-based benzisothiazolone (BIT) inhibitors of HLE.[6] The noteworthy features of this class of inhibitors are their specificity and incorporation of a built-in mechanism for regeneration of the enzyme activity (Figure 1).

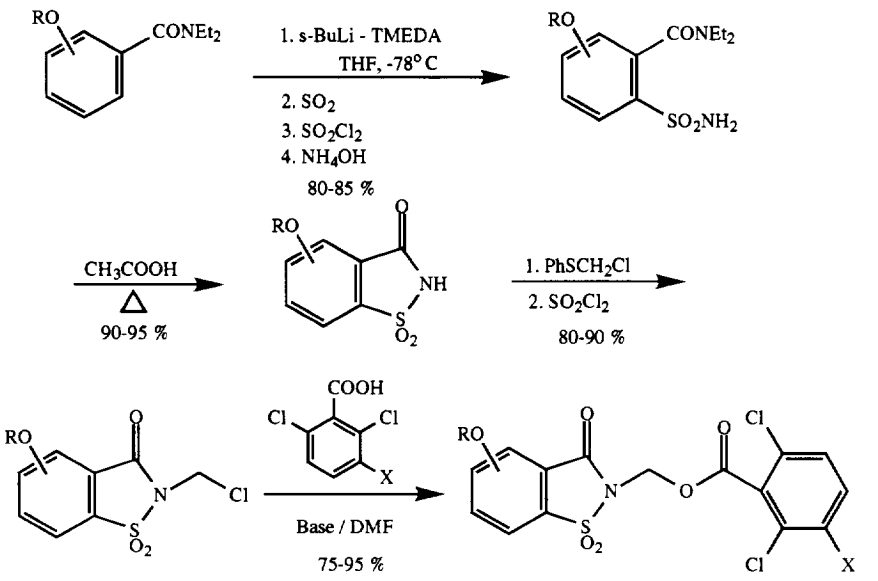
Figure 1. Proposed Mechanism of HLE Inhibition



In a previous publication, the effect of a small lipophilic substituent (e.g. an isopropyl group) at the 4 position of the BIT nucleus on the potency of HLE inhibitors was highlighted.[6]

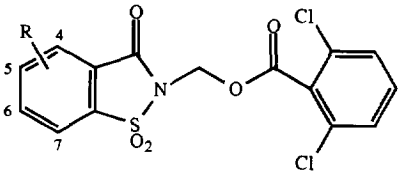
In the present article we report structure-activity relationship (SAR) studies with alkoxy substituents. In addition, we also demonstrate the *critical need* for alkoxy subtituents on the BIT nucleus to improve the human blood stability of these inhibitors. This is one of the important factors in developing an orally bioavailable therapeutic agent, since the rapid destruction of a drug in human blood would result in inadequate parent drug levels. We systematically introduced alkoxy group(s) at each of the four available positions on the BIT nucleus and determined the effects of steric and electronic factors on potency and stability. Compounds **1-12** were synthesized according to the general scheme outlined in Scheme 1.[7]

Scheme 1



Selection of the 2,6-dichlorobenzoate leaving group was based on its ability to confer superior potency.[8] As shown in Table 1, the ethoxy group was found to enhance potency relative to H or isopropoxy as a C-4 substituent. Comparison of the 4-H analog **1** to the 4-ethoxy analog **2** shows a 35-fold increase in the inactivation rate and a doubling of the reactivation rate. The net outcome of these two rates resulted in a 16-fold improvement in the apparent binding constant K_i^*. Compared to **2**, the 4-isopropoxy substituent in compound **3** decreased the inactivation rate 1.75 fold whereas the reactivation rate remained practically unchanged. In the case of compound **4**, introduction of 4,5-dimethoxy substituents increased the inactivation rate 2-fold compared to the 4-H analog **1**, thus reflecting a marginal improvement in potency. In compound **6**, substituting 6-H with an electron-donating methoxy group resulted, as expected, 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 5-fold in comparison to compound **2** resulting in a net 3.5-fold improvement in potency. In addition to the electronic factors, the superior potency of compound **6** may also be explained by hydrogen bond formation between the C-6 alkoxy group and valine ^{216} NH of the elastase.

Table 1. Effect of Alkoxy Substitution on HLE Inhibition.



HLE Inhibitory Activity [a]

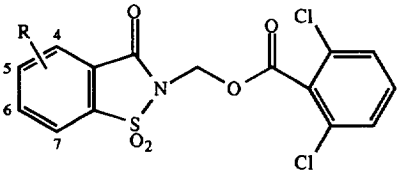
Cmpd	R =	$k_{inact}(M^{-1}sec^{-1})$	$k_{react}(sec^{-1})$	K_i^* (nM)
1	H	24,500	0.000066	2.7
2	$R^4 = OCH_2CH_3$	870,000	0.00015	0.17
3	$R^4 = OCH(CH_3)_2$	500,000	0.00012	0.24
4	$R^4, R^5 = OCH_3$	52,000	0.000062	1.2
5	$R^4, R^6 = OCH_3$	375,000	0.00003	0.08
6	$R^4 = OCH_2CH_3$ $R^6 = OCH_3$	580,000	0.000028	0.049
7	$R^4 = OCH_2CH_2CH_3$ $R^6 = OCH_3$	120,000	0.000041	0.34

(a) See ref. 6 for methodology. The potency of inhibition is expressed as an apparent binding constant, K_i^*, where $K_i^* = k_{react} / k_{inact}$. We have synthesized 4-methoxy and 4,7-dimethoxy analogs with the 1-phenyl mercapto tetrazole (PMT) leaving group, and they have similar potency as the 4-H derivative. We have observed parallel SAR between 2, 6-dichlorobenzoate and PMT leaving groups.

A computer modeling study of docking various 4,6-position substituted benzisothiazolones into the active site of HLE indicated the proximity of valine ^{216} NH and the C-6 alkoxy group with the H--O distance being about 3Å. With the 4-propoxy-6-methoxy analog **7**, the inactivation rate decreased 4.8-fold compared to compound **6** resulting in a 7-fold decrease in potency. Thus, as seen from the data presented in Table 1, the inactivation rates (k_{inact}) and apparent binding constants (K_i^*) of compounds **1-7** vary 35 fold, but the reactivation rates (k_{react}) vary less than 5 fold. Compound **2** has poor stability in human blood with a half life ($t_{1/2}$) of only 10 minutes. The major metabolite in blood is expected to be the 4-ethoxybenzisothiazolone.[9] A probable cause of poor stability in human blood may be the high reactivity of **2** towards esterases. There are two possible sites for esterase attack on compound **2**, the benzisothiazolone carbonyl and the carbonyl group of the 2,6-dichlorobenzoate leaving group. It is difficult to determine which of the two carbonyl groups of compound **2** is attacked by the esterases. Based on literature precedent, one could hypothesize the point of attack to be the benzisothiazolone carbonyl,[10] since approach of any nucleophile to the benzoate carbonyl is severly hindered by the ortho flanking 2,6-dichloro substituents. If this is indeed the case, then one can also hypothesize that either increasing the electron density or the steric hinderance around the BIT carbonyl group would reduce cleavage by

esterases. As shown in Table 2, substituting 6-H with an electron-donating ethoxy group in compound **8** resulted in *10-fold* improvement in stability in human blood as compared to compound **2.** However, as seen from the blood stability data of compound **9** and compound **2,** neither the steric nor the electronic factor alone can significantly improve the blood stability of these inhibitors. *Both* electronic and steric factors are crucial and their combined effects contribute positively towards improving the blood stability as seen in the analogs **8** and **5.**[11]

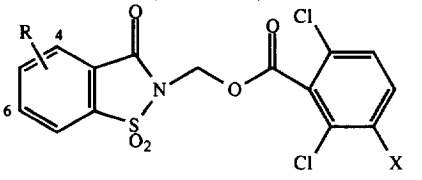
Table 2. Effect of Alkoxy Substitution on Stability in Human Blood

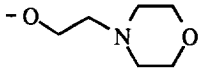
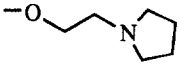
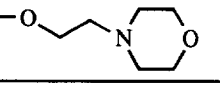


Cmpd	R =	HLE K_i* (nM)	Human Blood $t_{1/2}$ (Min)
2	$R^4 = OCH_2CH_3$	0.17	10
8	$R^4, R^6 = OCH_2CH_3$	0.08	98
5	$R^4, R^6 = OCH_3$	0.08	30
9	$R^6 = OCH_3$	0.47	5

Compound **8** could not be evaluated in an elastase-induced pulmonary hemorrhage model in hamster[12] due to its aqueous insolubility despite superior in vitro potency and stability. To overcome insolubility, we synthesized analogs **10-12.** These efforts were rewarded not only with in vivo active inhibitors, but also resulted in improved in vitro inhibitory potency against HLE (Table 3).

Table 3. In Vivo Activity of Alkoxy Substituted BIT Analogs



Cmpd	R =	X =	K_i* (nM)	In Vivo HLE Activity % Inhb. at 10 mg/kg
10	$R^4, R^6 = OCH_2CH_3$		0.07	51
11	$R^4, R^6 = OCH_2CH_3$		0.11	82
12	$R^4 = OCH_2CH_3$ $R^6 = OCH_3$		0.022	85

In summary, we have developed very potent, stable and in vivo active inhibitors of human leukocyte elastase. We have also identified the electronic and steric parameters responsible for improving the potency and stability in human blood. **WIN 68769 (12)** with a K_i^* = 0.022 nM is the most potent inhibitor synthesized in this class of HLE inhibitors.

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9. Unpublished results from these laboratories.
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