Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 4, pp. 331-336, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00030-5

THE DESIGN OF POTENT AND STABLE BENZISOTHIAZOLONE INHIBITORS OF HUMAN LEUKOCYTE ELASTASE

Dennis J. Hlasta,*† Malcolm R. Bell,† John J. Court,† Kenneth C. Cundy,‡ Ranjit C. Desai,†
Edward W. Ferguson,¥ Robert J. Gordon,¥ Virendra Kumar,† Alan L. Maycock,§
and Chakrapani Subramanyam†

Departments of Medicinal Chemistry,† Pharmaceutical Sciences,‡ Pharmacology,¥ and Biochemistry,§
Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Inc., 1250 South Collegeville Road, Collegeville, Pennsylvania 19426

Richard P. Dunlap, Shari L. Eiff, Catherine A. Franke, Albert J. Mura, and Anne G. Rowlands

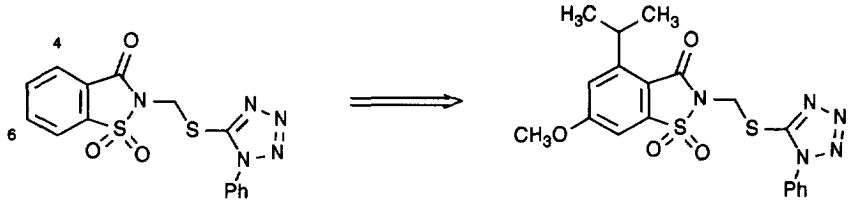
Kodak Research Laboratories, Eastman Kodak Company, Rochester, New York 14650.

Abstract: The lead compound for this SAR study, benzisothiazolone **1a,** was a 15 nM inhibitor of HLE, but was unstable in human blood ($t_{1/2}$<5 min). The introduction of lipophilic substituents at the R_4-position such as ethyl or isopropyl and modulation of the electrophilicity of the benzisothiazolone carbonyl led to the identification of a potent (K_i*=0.27 nM) and blood stable ($t_{1/2}$=260 min) inhibitor **2e**, WIN 63395.

Human leukocyte elastase (HLE) is a serine protease that has been proposed to be a primary mediator of pulmonary emphysema. An imbalance between HLE and the endogenous regulatory proteins, e.g., α_1-protease inhibitor, is postulated to occur in emphysema, where a deficiency of the α_1-protease inhibitor leads to elastin connective tissue destruction in the lung by HLE.[1] In other pulmonary diseases, such as cystic fibrosis[2] and adult respiratory distress syndrome,[3] an excess of HLE may overcome the protective effect of the endogenous inhibitors. An inhibitor of HLE should prove useful in the treatment of these pulmonary diseases.

The identification of mechanism-based inhibitors of HLE that display in vivo activity by the parenteral or oral route of administration has been difficult to achieve and has been attributed to poor hydrolytic and metabolic stability.[4] Mechanism-based inhibitors are by design reactive species that inactivate an enzyme by a suicide-like mechanism. The task to build into a molecule both inhibitory potency and metabolic stability has been difficult. Recently, orally active and bioavailable inhibitors of HLE have been described.[5,6,7] The first significant class of orally active compounds reported were the β-lactam, mechanism-based inhibitors.[5] Many SAR studies on the β-lactam series were required to identify the orally active inhibitor L-680,833. An important property that was improved and which led to orally active compounds was hydrolytic and blood stability.[5,8] This paper describes the design process by which we built into our mechanism-based inhibitors both inhibitory potency and blood stability. HLE inhibitors with in vivo activity were identified through our SAR study.

We have previously described the benzisothiazolone class of HLE inhibitors, the SAR at the C4-position and the proposed mechanism of inhibition.[9] The unsubstituted analogue **1a** was a 15 nM inhibitor, but was unstable in rat and human blood ($t_{1/2}$ < 5 min). It was therefore very unlikely that a simple analogue of **1a** would have oral activity or be orally bioavailable. Inspection of a computer model[10] of **1a** bound as a tetrahedral complex at the active site of HLE suggested that substituents at the C4 position would interact with the S1 specificity pocket. An ethyl or isopropyl at C4 appeared to provide an optimum fit with the hydrophobic S1 pocket. Additionally, it was hoped that a bulky group at C4 would block the addition of other nucleophiles to the carbonyl group of the benzisothiazolone thereby improving stability. The instability was thought to be due to an enzyme catalyzed hydrolysis, since the degradation of compound **1a** in human blood was very rapid ($t_{1/2}$ <5 min), while **1a** had a half-life of 500 min in a pH 7.8 phosphate buffer. Due to this large difference in rate, we assumed that the instability was due to hydrolysis by an enzyme, probably an esterase. Esterases are the major source of metabolism in serum and hydrolysis of ester or carbamate bonds of certain drugs can be very rapid.[11] To improve blood stability, we designed analogues by introducing substituents on the phenyl ring of **1a** that would maximize binding and inhibition of HLE based on our computer model and that would block addition of other nucleophiles to the benzisothiazolone carbonyl. Additionally, modulating the electrophilicity of the carbonyl by substitution on the phenyl could lead to analogues with improved stability, though it was not clear what effect this would have on HLE inhibitory potency. Electron donating groups should slow the rate of nucleophilic addition to the carbonyl, leading to less potent inhibitors with better stability.

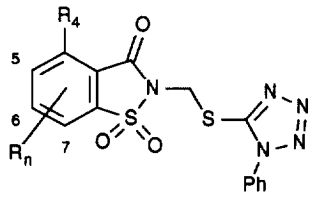


1a
HLE, K_i^* = 15 nM
human blood $t_{1/2}$ < 5 min

2e
HLE, K_i^* = 0.27 nM
human blood $t_{1/2}$ 260 min

The compounds **1b-1e** [12] had similar blood stability with half-lives of about one hour, and they were significantly more stable than the unsubstituted benzisothiazolone **1a** with the 4-isopropyl compound **1d** being the most potent (Table 1). Since these inhibitors possess a reactive carbonyl center, one could have expected inhibitory activity to correlate with instability, particularly if these inhibitors simply acylate the active site of HLE. However, neither the inactivation rate (k_{on}) nor the apparent binding constant (K_i^*) correlate in this series with blood stability half-life. In fact, the more potent **1d** is considerably more stable than the less potent **1a**. We propose that the improved blood stability of **1d** is due to the isopropyl group which sterically hinders the attack of esterases on the carbonyl group of the benzisothiazolone and deactivates the inhibitor to hydrolysis. Recently, a similar observation was made in the monocyclic β-lactam elastase inhibitors series. The introduction of ethyl groups adjacent to the carbonyl of the β-lactam gave potent inhibitors with improved stability presumably due to the hindrance of attack by nucleophiles on the β-lactam carbonyl.[5]

Table 1. HLE Inhibition and in vitro Stability of Benzisothiazolones 1 and 2:



Cmpd	R_4 =	R_n =	HLE inhibition [a]			human blood stability [b]
			k_{on} ($M^{-1}sec^{-1}$)	k_{off} (sec^{-1})	K_i^* (nM)	$t_{1/2}$ (min)
1a	H	H	5,600	0.000084	15	< 5
1b	CH_2CH_3	H	63,200	0.00013	2.0	54
1c	$CH_2CH_2CH_3$	H	100,00	0.000070	0.7	55
1d	$CH(CH_3)_2$	H	94,000	0.000028	0.3	45
1e	$CH(CH_3)(CH_2CH_3)$	H	94,000	0.000056	0.6	58
1f	$CH(CH_2CH_3)_2$	H	4,400	0.00026	60	100
1g	$C(CH_3)_3$	H	--	--	> 2,000	>> 120 [c]
2a	OCH_3	H	5,600	0.000076	14	
2b	OCH_3	5-OCH_3	53,800	0.000108	2.0	
2c	OCH_3	6-OCH_3	49,000	0.000029	0.6	
2d	OCH_3	7-OCH_3	1,100	0.000016	14.5	
2e	$CH(CH_3)_2$	6-OCH_3	44,500	0.000012	0.27	260
2f	$CH(CH_3)_2$	5,6-OCH_3	11,800	0.000061	5.2	65
2g	$CH_2CH_2CH_3$	5,6-OCH_3	240,000	0.000047	0.2	37

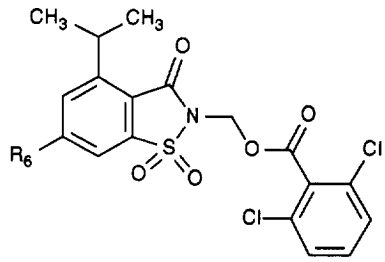
[a] The apparent binding constant is defined as $K_i^* = k_{off} / k_{on}$.[13] Methods are described in ref. 9. The binding constants and rates are reproducible to within ±10%. [b] Incubations were at 37 °C and disappearance of parent was monitored by HPLC. The half-lives are reproducible to within ±25%. [c] 88% of parent **1g** remained after incubation for 120 min.

The SAR study of the dimethoxy-benzisothiazolones **2a**-**2c** led to a potent and even more stable compound (Table 1). The 4,7-dimethoxy compound **2d** was prepared and tested. The compound **2d** was as potent as **1a** and **2a**, however, the electron donating 7-methoxy group of **2d** modulated the compound binding affinity for HLE, since the inactivation and reactivation rates were both five times slower than **2a**. The remaining dimethoxy compounds **2b** and **2c** were prepared and were shown to inactivate HLE ten times faster than **2a** (see k_{on}values in Table 1) . The 4,6-dimethoxy compound **2c** was the most potent dimethoxy derivative and had a desirably slow reactivation rate (k_{off} = 0.000029 s^{-1}). This SAR finding, combined with our hypothesis that electron donating substituents at C6 would improve blood stability, predicts that the most potent and stable analogue should be **2e**. Inhibitor **2e** bears the 4-isopropyl group which is important for inhibitory potency and stability and the 6-methoxy which should improve stability further. Indeed, this hypothesis proved to be correct. The

analogue **2e** was potent with a K_i* of 0.27 nM and was considerably more stable with a half-life of over four hours in human blood at 37 °C.

The electron donating capability of the 6-methoxy in **2e** should slow the rate of nucleophilic addition to the carbonyl. This suggests that the compound would be less potent, but have better stability than **1d.** However, the analogue **2e** was equipotent to the 4-isopropyl analogue **1d** and was considerably more stable with a half-life of 260 min. The better than expected potency of **2c** and **2e** is likely due to the formation of a hydrogen bond with the 6-methoxyl oxygen in the active site channel of HLE. The computer model[10] of **2e** with HLE predicts that the NH of Val[216] forms a hydrogen bond with the oxygen of the 6-methoxy group. The O-H-N distance of 3.2 Å is within the typical range of 2.6-3.3 Å for hydrogen bonds in proteins.[14] To further sterically crowd the carbonyl, an additional 5-methoxy substituent was introduced in the analogues **2f** and **2g**. The buttressing effect of 5-methoxy on the 4-alkyl substituent should sterically hinder the carbonyl; however, these analogues were either less potent (**2f**) or less stable (**2g**) than **2e**. Krantz has examined the effect of electron donating groups on hydrolytic stability in a series of benzoxazinone HLE inhibitors. Electron donating groups did improve the hydrolytic stability of these alternate substrate inhibitors; however, subnanomolar inhibitory potency was not retained when stability was improved.[15]

Table 2. *R_6 Substituent Effects on HLE Inhibition and Stability:*



Cmpd	R_6 =	HLE inhibition			human blood stability
		k_{on} ($M^{-1}sec^{-1}$)	k_{off} (sec^{-1})	K_i^* (nM)	$t_{1/2}$ (min)
3a	H	900,000	0.000027	0.03	30
3b	F	1,000,000	0.000030	0.03	< 15
3c	OCH_3	522,000	0.000012	0.023	140
3d	NMe_2	12,300	0.000027	2.2	--
3e	1-methyl-4-piperazinyl	21,500	0.000024	1.1	110

We have found that the 2,6-dichlorobenzoate (DCB) leaving group increases the potency about 10 fold over the corresponding phenylmercaptotetrazole (PMT) analogues,[16] while retaining blood stability (compare compounds

3a and **3c** with **1d** and **2e**). The effect of electron donating and electron withdrawing groups at C6 was explored in the DCB series (Table 2).[12] The compound **3b** containing the electron withdrawing 6-fluoro substituent was equipotent to the 6-hydrogen compound **3a**, but was much less stable, presumably due to the increased electrophilicity of the carbonyl in **3b**. The inhibitors **3d** and **3e** containing the electron donating 6-amino substituents were less potent than **3a**. The loss in electrophilicity of the carbonyl due to the strongly electron donating 6-amino accounts for the reduced inhibitory potency and improved stability of **3e**. The 6-methoxy derivative **3c** was the most potent (K_i^* = 0.023 nM) and stable ($t_{1/2}$ = 140 min) inhibitor in the DCB series.

In summary, we have designed potent, stable, mechanism-based inhibitors of HLE. The SAR study investigating HLE inhibitory potency and blood stability identified the 4-isopropyl-6-methoxy-benzisothiazolone nucleus as the optimum nucleus in this series. Two analogues, WIN 63395 (**2e**) and WIN 63394 (**3c**), were tested in the HLE-induced hemorrhage hamster model,[17] however, the results were variable. When hamsters were pretreated with 10 mg/kg, i.v. of **2e** in an oil-in-water emulsion, inhibition of 24 or 73% was observed in separate tests and with **3c** inhibition of 37 or 100% was observed. The inhibitors **2e** and **3c** were tested orally at 100 mg/kg in hamsters, but only weakly inhibited (36% and 24%, respectively) the HLE-induced hemorrhage. In dogs the oral bioavailability of **2e** was only 2%. The variable results obtained with **2e** and **3c** in the i.v. hamster model is attributed to their physical properties. These compounds are very lipophilic and absorption may be dissolution limited, since **2e** and **3c** are also very insoluble in water. This problem has been addressed by the incorporation of aqueous solubilizing groups on the leaving group portion of the DCB inhibitor **3c** and has led to the identification of orally bioavailable compounds.[18] This benzisothiazolone series may represent a new class of therapeutic agents for the treatment of pulmonary diseases such as emphysema, cystic fibrosis, and ARDS.

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(*Received in USA* 28 *October* 1994; *accepted* 16 *December* 1994)