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INHIBITORS OF HUMAN LEUKOCYTE ELASTASE. 3.[1] INHIBITION BY TETRAHYDROBENZISOTHIAZOLINYLMETHYL ARYL CARBOXYLATES.

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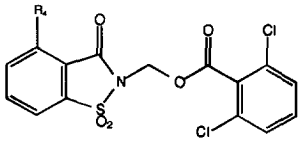
Abstract: Potent mechanism based inhibition of human leukocyte elastase (HLE) by tetrahydrobenzisothiazolones (**2**) is described. Structure activity relationships studies led to the identification of WIN 62816 (**2c**), the most potent inhibitor in this series with a $K_i^* = 0.7$ nM.

Human leukocyte elastase (HLE), a serine proteinase that is released by neutrophils at sites of inflammation, is believed to be the major causative factor in the etiology of many pulmonary disorders such as emphysema[2], acute respiratory distress syndrome[3] and chronic bronchitis.[4] Inhibitors of this enzyme should be of therapeutic potential in the treatment of these disorders.[5] Recently, we reported that benzisothiazolones (**1**) are potent and selective inhibitors of HLE and proposed a mechanism by which these compounds inhibit HLE.[6,7] In our efforts to expand the scope of this class of HLE inhibitors, we synthesized a number of tetrahydro analogs of **1**. In this paper we report that these tetrahydrobenzisothiazolones (**2**) are potent-mechanism based inhibitors of HLE.



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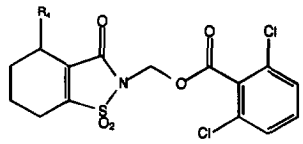

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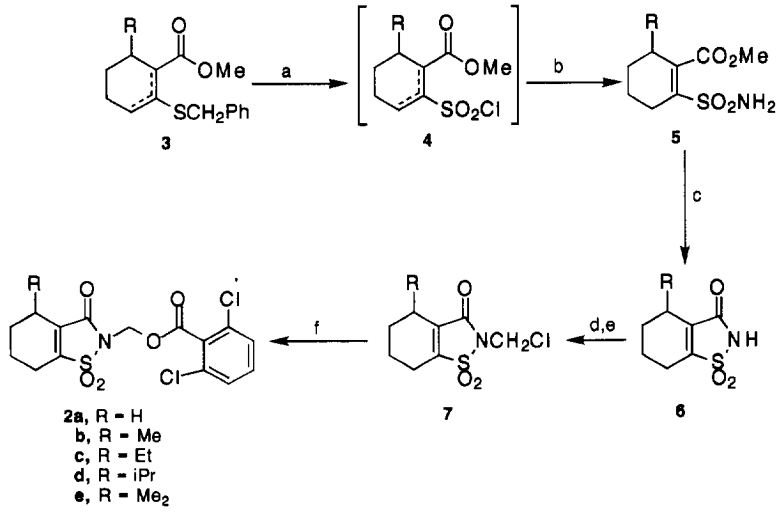
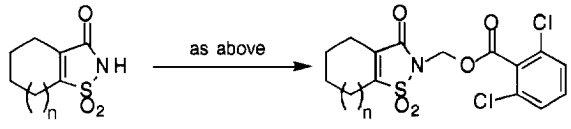
Chemistry: The target compounds 2 were prepared as shown in Scheme I. Reaction of the readily available vinyl sulfide **3**[8] with Cl_2 in $HOAc/H_2O$ led to efficient oxidative debenzylation,[9] providing the sulfonyl chlorides **4** in near quantitative yields. Treatment of 4 with 28% NH_4OH gave the mixture of sulfonamides **5**, which upon stirring in MeOH in the presence of NaOMe, led to the tetrahydrobenzisothiazolones **6**. Conversion of **6** to the

chloromethyl derivatives **7**, was uneventful under the conditions reported previously for the analogous benzisothiazolone nucleus.[7] Finally, alkylation of 2,6-dichlorobenzoic acid with **7** provided the benzoates **2** in excellent yields.[10] Synthesis of the cyclopentyl (compound **9a**) and cycloheptyl (**9b**) analogs was achieved from the readily available isothiazolones **8**.[11]

Scheme I

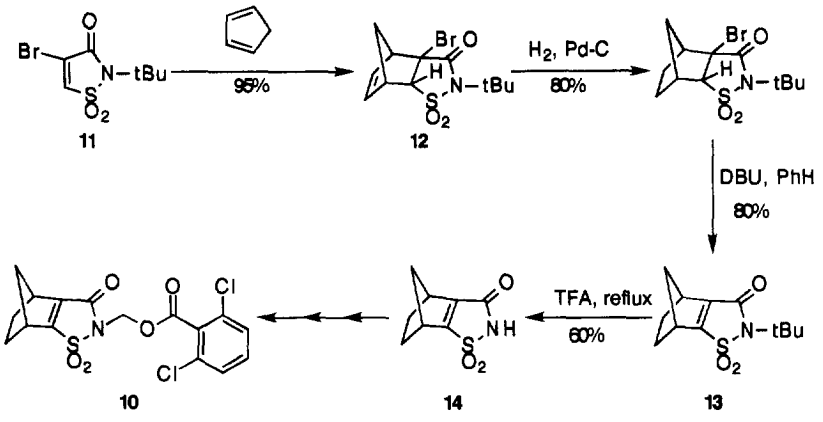


Reagents: (a) $Cl_2(g)/H_2O$ /HOAc, 80-90%; (b) aq.NH_3/THF; (c) NaOMe/MeOH, 70-80% from **6**
(d) $PhSCH_2Cl/nBu_4N^+Br^-$/Toluene, reflux, 70-80%; (e) SO_2Cl_2/CH_2Cl_2, r.t, 60-80%
(f) 2,6-Dichlorobenzoic acid/K_2CO_3/DMF/cat. $nBu_4N^+Br^-$, 65-70 °C, 50-60%



The bicyclo derivative **10** was prepared as shown in Scheme II. Thus Diels-Alder reaction of bromide **11**[12] with cyclopentadiene gave the adduct **12** in 95% yield. Initial attempts to eliminate elements of HBr from **12** led to complete decomposition of starting material. However, when the double bond in **12** was reduced (H_2, Pd-C), elimination of HBr to give the tetra substituted olefin **13** was easily achieved using DBU in benzene. Removal of the t-Bu protecting group (TFA, reflux) from **13** gave the isothiazolone **14** which was converted to the target dichlorobenzoate **10** as described above for other targets.

Scheme II



Biological results and discussion: The HLE inhibitory activity of the dichlorobenzoates **2a-e**, **9a-b** and **10** is shown in Table 1. As seen, introduction of small lipophilic groups at C-4, led to improvement in inhibitor potency. This is consistent with modeling studies, and also with the SAR observed for our benzisothiazolone inhibitors **1**.[6] However, the SAR for different R4 substituents does not parallel among the two series. For eg: The 4-methyl compound **2b**, showed a 18 fold improvement in potency vs the 4-hydrogen analog **2a** where as in the case of benzisothiazolones **1** such modification led to no improvement in activity. Compounds **2d,e** were as potent as **2b**, inspite of 8 and 5 fold loss in their inactivation (k_{inact}) rates. This is because their reactivation rates (k_{react}) also decreased proportionately. This is contrary to what has been observed among the benzisothiazolones **1**. Here, the isopropyl analog (**1**, R4 = i-Pr) was 100 fold more potent than the corresponding methyl derivative (**1**, R4 = Me).

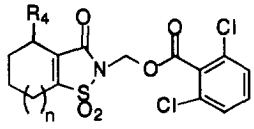
In order to better understand this discrepancy, computer modeling studies were perfomed.[13] Although the potency ($K_i^* = k_{react}/k_{inact}$) for HLE inhibition by this class of inhibitors is a combination of the following events: initial binding, covalent modification and subsequent hydrolysis of the enzyme inhibitor complex, the proposed mechanism[6,7] requires that each of these inhibitors have similar rates of reactivation (k_{react}). The rates of inactivation (k_{inact}) should then be the major determinant of HLE inhibitory activity of these compounds. The data shown in Table 1 wherein the k_{inact} vary by more than 25 fold but the k_{react} vary by less than 5 fold is consistent with this hypothesis. The rates inactivation of HLE (and hence the potency K_i^*) by these inhibitors would be largely dependent on the nature of the R4 group and their binding interaction with the S1 specificity pocket of HLE.[7] Hence, we postulated that there should be a correlation between the observed binding affinity (-log K_i^*) and the computed relative enthalpy ($\Delta\Delta H$) of interaction. The later was determined as follows. The two series of inhibitors (compounds **1** and **2**) were docked into the X-ray crystal structure of HLE,[14] such that the R4 substituent is bound to the S1 specificity pocket of HLE[15] and the carbonyl of the benzisothiazolone moiety is constrained into the oxyanion hole by covalently linking it with Ser[195]. The combined system was minimized

keeping the backbone C-α, N and C atoms fixed and the enthalpy of binding was calculated on the basis of the simple equilibrium:

$$E + L = EL$$
$$\Delta H = H_{EL} - (H_E + H_L)$$

where EL is the covalently bonded complex between the enzyme $Ser^{195}OH$ and the isothiazolone carbonyl of the ligand. The relationship between observed binding affinity (-log K_i^*) and the computed relative enthalpy of interaction is shown in Table 2. As evident from the data, this modeling effort correctly predicted the rank order potency for R_4 substituents in the tetrahydroderivatives **2** to be Et > Me > *i*-Pr. However, among the benzisothiazolones (**1**) series, the predicted rank order was not in agreement with the observed values. This suggests that the interaction enthalpy , uncorrected for hydrophobic and electronic interactions is just a qualitative representation for observed K_i^*. More refined calculations which takes into account the hydrophobic and electronic interactions are needed. More important would be the availability of the crystal structure of HLE-benzisothiazolone inhibitor complex.

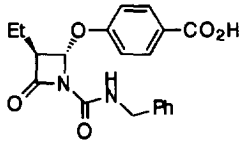
Table 1: HLE inhibitory activity of tetrahydrobenzisothiazolones:



Compd.	n =	R4 =	HLE activity[a] k_{inact} (M^{-1} sec^{-1})	HLE activity[a] k_{react} (sec^{-1})	K_i^* (nM)
1	1	i- Pr	900,000	0.000027	0.03
2a	1	H	3,300	0.000059	18
2b	1	Me	36,000	0.000036	1
2c	1	Et	83,000	0.000058	0.7
2d	1	i-Pr	10,000	0.00001	1
2e	1	4,4-Me_2	18,500	0.000018	1
9a	0	--	ND	ND	5
9b	2	--	ND	ND	100
10	--	--	ND	ND	0.8
ICI-200,355		---	94,000	0.000037	0.4

[a]HLE inhibitory activity was determined as described in ref.7 and the rates and binding constants were reproducible to within ±10%. ND: Not determined.

The 4-ethyl compound **2c** with a K_i^* = 0.7 nM was the most potent inhibitor in this series and its stoichiometry for HLE inhibition was 2:1, suggesting that one of the enantiomers of **2c** is significantly more potent than the other. This would be in line with the observation by Merck group, who have shown that one of the enantiomers of the trans 3,4-disubstituted azetidinone **15**, was 10 fold more potent than the other.[16]



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The rate of inactivation and the HLE inhibitory activity of **2c** is similar to the known transition state inhibitor **ICI-200,355**.[17] Among compounds (**2a, 9a-b**) with different carbocyclic rings attached to the benzisothiazolone nucleus, the cyclopentyl analog **9a** was the most potent. It is conceivable that the ring strain associated with the 5,5 system could make the isothiazolone carbonyl group of **9a** more susceptible for attack by ser^{195} of HLE, leading to the enhanced activity. The conformationally restricted bicyclo derivative **10** was as active as **2c**, the most potent inhibitor in this series.

Table 2. The observed binding affinity $\log K_i^*$ and computed relative enthalpy of interaction.

Compound	R =	-log Ki*	ΔΔH
1a	H	8.70	0.00[a]
1b	Me	8.70	-4.73
1c	Et	10.15	-9.11
1d	i- Pr	10.52	-1.92
2a	H	7.74	0.00[a]
2b	Me	9.00	-2.77
2c	Et	9.15	-7.30
2d	i - Pr	9.00	3.01

[a]The value of H_{EL}-H_L for this reference compound was -345.94 kcal/mole.

In summary, tetrahydrobenzisothiazolinyl benzoates **2** have been discovered to be potent mechanism-based inhibitors of human leukocyte elastase. The 4-ethyl analog, WIN 62816 (**2c**) is the most potent inhibitor in this new class of HLE inhibitors with a Ki* = 0.7 nM. The arylcarboxylate leaving group and the cycloalkyl portion of **2** offers an opportunity for considerable variation as a design element which could lead to enhanced potency among this class of HLE inhibitors.

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References and Notes:

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