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A COMPARATIVE SAR AND COMPUTER MODELING STUDY OF BENZISOTHIAZOLONE, MECHANISM-BASED INHIBITORS WITH PORCINE PANCREATIC AND HUMAN LEUKOCYTE ELASTASE[1]

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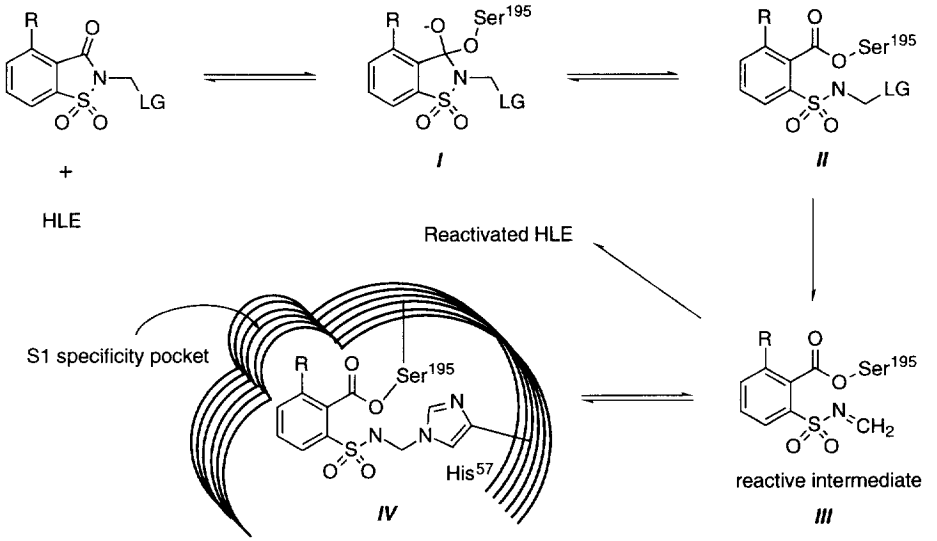
Abstract: Distinct differences in the SAR for HLE and PPE inhibition in this class of compounds were observed. For example, larger lipophilic substituents at the benzisothiazolone 4-position afforded inhibitors that were potent against HLE, but inactive against PPE. These findings are consistent with computer models of inhibitor-enzyme complexes built using the X-ray structure coordinates of HLE and PPE. These models show that substituents at the benzisothiazolone 4-position fit into the S1 specificity pocket of the enzyme and that other differences in the SAR can be explained based on the structural differences of HLE and PPE.

Human leukocyte elastase (HLE) is a serine protease that is found in the granules of neutrophils. HLE has been proposed to be a primary mediator of emphysema,[3] thus inhibitors of HLE should prove useful in the treatment of emphysema. Inhibitors of HLE may also find use in other pulmonary diseases such as adult respiratory-distress syndrome,[4] cystic fibrosis,[5] and chronic bronchitis,[6] where elastase has been implicated as a causative agent or contributory to symptoms. The benzisothiazolones are a class of mechanism-based HLE inhibitors that have been reported to react by a suicide mechanism to cross link the enzyme active site Ser^{195} and His^{57}.[7] Structure-activity relationship (SAR) studies on the benzisothiazolone class of elastase inhibitors have previously been described. These studies were based on computer modeling studies that have as yet not been disclosed. The SAR studies for the optimization of inhibitor potency[7] and in vitro metabolic stability[8] were key in the discovery of orally bioavailable benzisothiazolone inhibitors of HLE.[9] The computer modeling studies described in this paper were critical for the optimization of the potency and stability of these inhibitors that led to the discovery that the 4-isopropyl-6-methoxy-benzisothiazolone nucleus was optimum for inhibitor potency, in vitro metabolic stability, and selectivity of inhibition.[9]

The *N*-arylbenzisothiazolones were known alternate substrate inhibitors of HLE that were proposed to acylate the active site Ser^{195}.[10] Based on the mechanism of serine proteases[11] and on the chemical reactivity of the related *N*-acetoxymethyl-benzisothiazolone,[12] the benzisothiazolone inhibitors were postulated to inactivate elastase by the mechanism-based inhibition pathway shown in Scheme 1. The first compound tested in this series was *N*-acetoxymethyl-benzisothiazolone (K_i* = 100 nM).[7] Computer modeling of this inhibitor cross-linking the PPE active site gave an acceptable model with reasonable bond angles and lengths. The model showed that the R_4 benzisothiazolone substituent would interact with the S1 specificity pocket and predicted that a small lipophilic group such as ethyl would be optimum for PPE. Modeling studies using

X-ray coordinates for HLE later showed that larger R_4 substituents could be accommodated, since the S1 pocket was larger than the PPE S1 subsite.[13] Therefore, the synthetic chemistry was directed towards preparing 4-substituted benzisothiazolone analogs.[14] The HLE and PPE inhibition SAR for these analogs is described in this paper along with the computer models for the enzyme-inhibitor complex. The differences in the SAR of HLE and PPE inhibition are rationalized based on computer models of enzyme-inhibitor complexes and are consistent with the proposed mechanism of elastase inhibition shown below.

Scheme 1. ***Proposed Mechanism of Inhibition.***



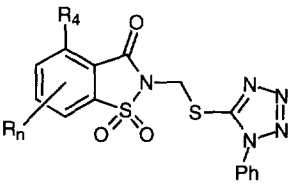
We had previously reported that substitution at the 4-position of the benzisothiazolone led to improved inhibitory potency.[7] Consistent with a favorable hydrophobic interaction of the benzisothiazolone 4-substituent with the HLE S1 specificity pocket, the 4-isopropyl **5** was the most potent HLE inhibitor (Table 1). The S1 pocket of HLE is known to be larger than PPE and Leu and Val are the preferred P1 substituents in inhibitors or substrates.[13] The S1 pocket of PPE is smaller than HLE and Ala and Nva are the preferred P1 substituents.[13] Consistent with an interaction of the benzisothiazolone 4-position with the PPE S1 site, the most potent PPE inhibitors were the 4-ethyl and the 4-n-propyl compounds **3** and **4**, while the s-butyl **6** and the 3-pentyl **7** did inhibit HLE, but were inactive against PPE.

In our computer modeling studies, inhibitors were manually docked in the active site channel of the enzyme (HLE or PPE) using the molecular modeling program Quanta/Charmm. The X-ray structure of the HLE complex with MeOSuc-Ala-Ala-Pro-Ala-CH_2Cl (PDB1HNE)[15] in the Protein Data Bank was used for HLE modeling, while the X-ray structure of the native PPE (PDB3EST)[16] was used for the PPE modeling studies. When forming a covalent bond between the inhibitor carbonyl and Ser^{195}, only one orientation of the inhibitor in the active site channel was reasonable due to unfavorable non-bonded interactions between the enzyme and the inhibitor in all other orientations. The Ser^{195} hydroxyl of elastase was bonded to the carbonyl carbon of the inhibitor and energy minimization of the enzyme-inhibitor complex gave model *I* (shown in Scheme 1 and Figures 1 and 2). The inhibitor alkoxide of the HLE model is bound in the oxyanion hole by

the NH of Gly^{193} and Ser^{195} (O-H-N distances of 2.6 Å and 3.0 Å, respectively) (see Figure 3). This binding motif is analogous to the binding mode for the tetrahedral intermediate of a substrate scissile amide bond in serine proteases.[13] Also, in this model the imidazole of His^{57} is posed above the nascent imine of the inhibitor and is in position to react with the electrophilic sulfonyl imine when generated. The model also maintains the hydrogen bond between the imidazole of His^{57} and the carboxyl of Asp^{102}, which is an important proton relay in the mechanism of amide bond cleavage by serine proteases and serves to remove or add a proton to the intermediates along the reaction coordinate to amide bond cleavage. The cross-linked active site model ***IV*** was obtained starting with the model ***I***. As shown in Scheme I, the C3-N2 ring bond of ***I*** was broken, the leaving group was removed, and a ~80 degree rotation about the Ar-S bond brought the imine carbon atom of ***III*** to within bonding distance of the epsilon N of the His^{57} imidazole. The imine CH2 to imidazole N bond was formed and energy minimization gave the model ***IV*** shown in Figure 3. The tetrahedral intermediate model ***I*** and the active site cross-linked model ***IV*** of inhibitor **12** have very similar geometries. When the cross-linked model ***IV*** is formed, the His^{57} imidazole moved <0.5 Å towards the inhibitor, but was still within H-bond distance from the Asp^{102} carboxylate. This emphasizes the degree of inherent fit that the benzisothiazolones have for the active site of HLE. In models ***I*** and ***IV*** the aromatic ring and its substituents were nearly identical, therefore, to simplify the modeling experiments, the tetrahedral intermediate model ***I*** was used for all other examples. Models ***I*** and ***IV*** have limited degrees of freedom which simplify the modeling experiments, while providing meaningful results. The intermediates ***II*** and ***III*** are difficult to model due to the large number of accessible conformations and therefore were not used in these studies.

PPE Modeling. The tetrahedral intermediates of **3**, **10**, and **12** were modeled in the active site of PPE and the overlaid structures are shown in Figure 1. The 4-ethyl benzisothiazolone **3** was the most potent of the 4-alkyl substituted inhibitors of PPE (see **1-5** in Table 1). The 4-ethyl of **3** fills the PPE S1 pocket and substituents much larger than ethyl would result in repulsive van der Waals interactions with the protein surface or the inhibitor would be displaced from the protein surface. Indeed, a 4-isopropyl substituent displaces the inhibitor away from the protein surface, but apparently binds sufficiently so that inhibitor **5** is only 1.5 times less potent than **3** (cf. **12** in Figure 1). However, substituents larger than isopropyl result in complete loss of inhibitor potency. The 4-s-butyl and the 4-(3-pentyl) compounds did not inhibit PPE (see **6** and **7** in Table 1). The inhibitor **10** was the most potent PPE inhibitor of the alkoxy-substituted benzisothiazolones **8-12**. This result can be explained by the formation of a H-bond between the 6-methoxy oxygen of **10** and the NH of Val^{216}. Indeed, the 6-methoxy oxygen is within hydrogen bonding distance of the Val^{216} NH (O-H-N distance is 3.0 Å), since the S1 pocket can accommodate the 4-methoxy group (Figure 1). However, the 4-isopropyl of **12** causes displacement of the inhibitor away from the active site, such that the 6-methoxy is not within hydrogen bonding distance to the NH of Val^{216}(O-H-N distance is 3.8 Å). These modeling results are consistent with the PPE inhibition data shown in Table 1 and explain the potency of **10** and the inactivity of **12**. The 4-methoxy compound **8** is less potent than the carbon analog **3** presumably since alkoxy is less lipophilic than alkyl, and the alkoxy group does not solvate the lipophilic surface of the S1 pocket as well as the alkyl group. A 5-methoxy substituent based on the model shown in Figure 1 would lead to a unfavorable steric interaction with the protein surface. This explains the fact that the 4,5-dimethoxy analog **9** was inactive against PPE. We do not have a good explanation for the inactivity of the 4,7-dimethoxy analog **11** against PPE, since the 7-position points into solvent and should have little effect on binding.

Table 1. *Enzyme Inhibition Data.*[14]



Cmpd	Substitution R_4 =	R_n =	HLE [a, b] k_{on}, $M^{-1}s^{-1}$	HLE [a, b] K_i^*, nM	PPE [a] k_{on}, $M^{-1}s^{-1}$	PPE [a] K_i^*, nM
1	H	H	5,600	15	240	62
2	CH_3	H	3,200	32	280	74
3	CH_2CH_3	H	63,000	2.0	1,300	20
4	$CH_2CH_2CH_3$	H	100,000	0.7	130	26
5	$CH(CH_3)_2$	H	94,000	0.3	140	39
6	$CH(CH_3)(CH_2CH_3)$	H	94,000	0.6		>10,000
7	$CH(CH_2CH_3)_2$	H	4,400	60		>1,500
8	OCH_3	H	5,600	13.6	210	400
9	OCH_3	5-OCH_3	54,000	2.0		>1,000
10	OCH_3	6-OCH_3	49,000	0.6	1,300	15
11	OCH_3	7-OCH_3	1,100	14.5		>10,000
12	$CH(CH_3)_2$	6-OCH_3	44,500	0.27		>1,000
	Methoxysuccinyl-Ala-Ala-Pro-Val-CH_2Cl		3,150 [c]	--	830 [c]	--

[a]The apparent binding constant is defined as $K_i^* = k_{off}/k_{on}$. Methods are described in ref 7. The binding constants and rates are reproducible to within ±10%. [b]Data taken from refs 7 and 8. [c]Irreversible inhibitor.

HLE Modeling. The tetrahedral intermediates of **1**, **3**, **5**, **9**, **10**, and **12** were modeled with HLE and the overlaid structures are shown in Figure 2 with key hydrogen bonds indicated. All inhibitors have the same geometry in the active site. In contrast to the PPE modeling results, the 6-methoxy of both **10** and **12** are within hydrogen bonding distance of the HLE Val^{216} NH (O-H-N distances are 3.2 Å), since the 4-isopropyl group is easily accommodated in the S1 pocket. A reciprocal H-bond between peptide inhibitors with Val^{216} is a key component of the ß-sheet binding motif of inhibitors with PPE.[13] The model of **12** with HLE is displayed with a side view of the HLE S1 pocket shown in green in Figure 4. The molecular surface of native PPE is overlaid and is shown in yellow. The 4-position of the benzisothiazolone is oriented toward the S1 pocket, and the 4-isopropyl of **12** fills the HLE S1 pocket, but intersects the PPE surface. This is consistent with the model of **12** with PPE (Figure 1) where the inhibitor is displaced away from the active site channel, such that the 6-methoxy is not within hydrogen bonding distance of the Val^{216} NH. The model in Figure 4 also shows that a small substituent at the 5-position can be accommodated in the HLE model, however, any

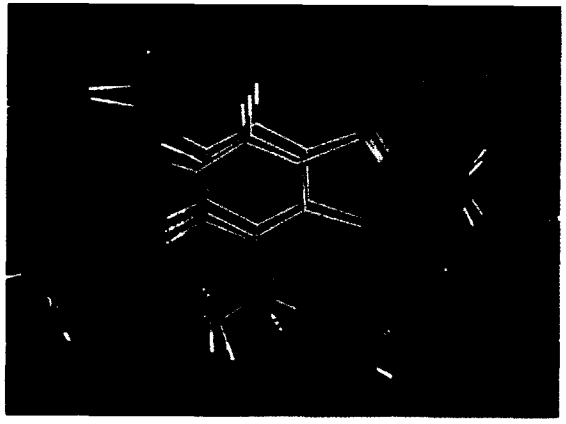


Figure 1. The Tetrahedral Intermediate Models I of Inhibitors **3**, **10** and **12** with PPE.

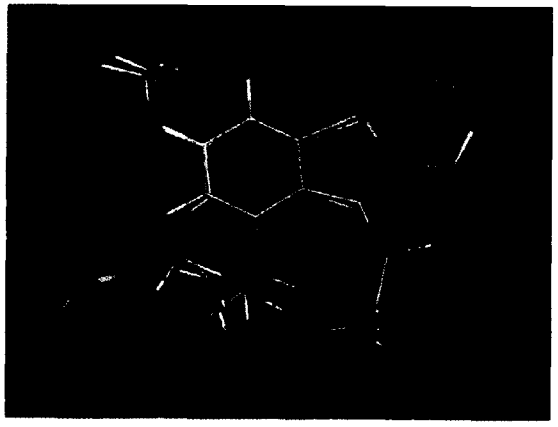


Figure 2. The Tetrahedral Intermediate Models I of Inhibitors **1**, **3**, **5**, **9**, **10** and **12** with HLE.

Figure 3. A Comparison of Models I (line) and IV (stick) of the HLE–Inhibitor Complex.

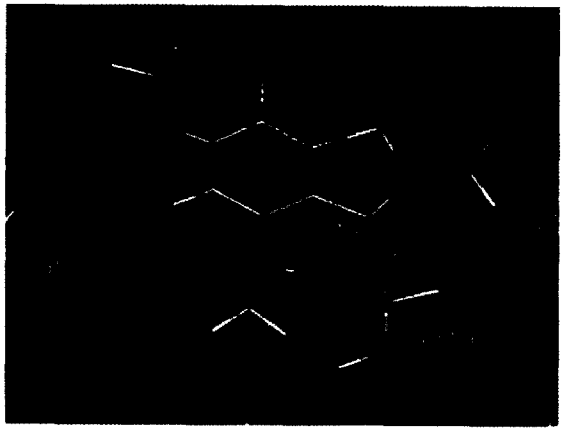


Figure 4. Model of HLE–Inhibitor Complex of **12** with HLE (green) and PPE (yellow) Surfaces.

substituent at the benzisothiazolone 5-position would intersect the PPE surface. This explains the result that the 4,5-dimethoxybenzisothiazolone **9** does not inhibit PPE, but is a potent HLE inhibitor.

The initial model of the active-site cross-linked enzyme-inhibitor complex predicted that small lipophilic substituents at the 4-position would provide potent elastase inhibitors.[7] The present study confirms that prediction and with the PPE and HLE inhibitory data, provides an explanation of the SAR results in terms of interactions at the enzyme active site. This study reemphasizes the fact that human sources of proteins are required in drug discovery, since the PPE SAR would have led to the selection of the inferior 4-ethyl benzisothiazolone for development, instead of the optimum 4-isopropyl-6-methoxybenzisothiazolones.[9]

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