Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 1543–1548

BIOORGANIC & MEDICINAL CHEMISTRY

Inhibition of Serine Proteases by Functionalized Sulfonamides Coupled to the 1,2,5-thiadiazolidin-3-one 1,1 Dioxide Scaffold

William C. Groutas,* Shu He, Rongze Kuang, Sumei Ruan, Juan Tu and Ho-Kit Chan

Department of Chemistry, Wichita State University, Wichita, KS 67260, USA

Received 26 June 2000; accepted 30 January 2001

Abstract—A challenge associated with drug design is the development of selective inhibitors of proteases (serine or cysteine) that exhibit the same primary substrate specificity, that is, show a preference for the same P_1 residue. While these proteases have similar active sites, nevertheless there are subtle differences in their S and S′ subsites which can be exploited. We describe herein for the first time the use of functionalized sulfonamides as a design and diversity element which, when coupled to the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold yields potent, time-dependent inhibitors of the serine proteases human leukocyte elastase (HLE), proteinase 3 (PR 3) and cathepsin G(Cat G). Our preliminary findings suggest that (a) appending to the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold recognition and diversity elements that interact with both the S and S′ subsites of a target protease may result in optimal enzyme selectivity and potency and, (b) functionalized sulfonamides constitute a powerful design and diversity element with low intrinsic chemical reactivity and potentially wide applicability.

Introduction

Several lines of evidence suggest that serine and metalloproteases released by neutrophil and macrophages play a pivotal role in the etiology and pathophysiology of inflammatory diseases such as, for example, pulmonary emphysema,[1–3] chronic bronchitis,[4] adult respiratory distress syndrome (ARDS),[5,6] cystic fibrosis,[7,8] and others.[9,10] Poor regulation of the activity of these enzymes due to depressed levels of their physiological protein inhibitors (alpha-1-proteinase inhibitor, secretory leukocyte protease inhibitor, human monocyte/neutrophil elastase inhibitor, elafin, tissue inhibitors of metalloproteases) can lead to the unrestrained destruction of the major components of the extracellular matrix and basement membrane. Low molecular weight compounds capable of controlling the activity of these enzymes, thereby redressing the protease/antiprotease imbalance, may lead to the emergence of new therapeutic agents.[11–14]

In recent studies we have described the structure-based design of a novel heterocyclic scaffold (1,2,5-thiadiazolidin-3-one 1,1 dioxide) and have demonstrated that the scaffold (a) is a general template suitable for the design of inhibitors of (chymo)trypsin-like serine proteases; (b) can be used to fashion inhibitors that show absolute selectivity between neutral, basic and acidic serine proteases; (c) is amenable to the construction of libraries for lead identification, and the rapid optimization of potency and enzyme selectivity, and (d) can be used to probe and exploit subtle differences in the active sites of closely-related serine proteases by allowing the attachment and optimal spatial orientation of recognition elements that permit the exploitation of favorable binding interactions with the S *and* S′ subsites of a target enzyme.[15–20] We describe herein the use and preliminary evaluation of sulfonamide moiety (**II**) as a powerful and versatile *design* and *diversity* element which, when coupled to the 1,2,5-thiadiazolidin-3-one 1,1 dioxide template, yields potent inhibitors (**I**) of the serine proteases human leukocyte elastase (HLE), proteinase 3 (PR 3) and cathepsin G (Cat G).

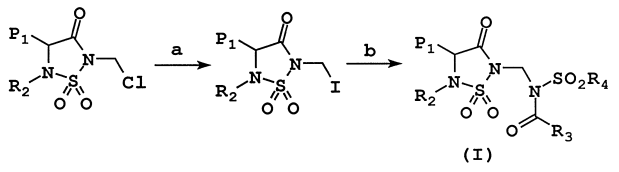
Results

Chemistry

Sulfonamide derivatives **1**–**17** were synthesized according to Scheme 1. The precursor sulfonamides (**II**) were synthesized as summarized in Scheme 2, by reacting an alkyl or aryl sulfonamide with a carboxylic acid in the presence of carbonyl diimidazole (CDI)[21] or an alkyl

*Corresponding author. Tel.: +1-316-978-3120; fax: +1-316-978-3431; e-mail: groutas@wsuhub.uc.twsu.edu

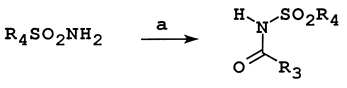
0968-0896/01/$ - see front matter
PII: S0968-0896(01)00037-2



(I)

[a]NaI/acetone; [b]$R_4SO_2NH(C{=}O)R_3$/DBU

Scheme 1. Synthesis of derivatives of (I): (a) NaI/acetone; (b) R_4SO_2NH (C=O) R_3/DBU.



(II)

When R_3 = alkyl or aryl, a = RCOOH/CDI/THF[21]

= OR, a = ClCOOR/TEA/DMAP[22]

= NHR or NHCH(R)COOR, a = O=C=NR or O=C=NCHRCOOR[23]

Scheme 2. Synthesis of precursor sulfonamide derivatives (II).

chloroformate in the presence of triethylamine and 4-dimethylamino pyridine (DMAP)[22] or an isocyanate in the presence of triethylamine.[23] Thus, the appropriate chloromethyl derivative was first reacted with NaI in dry acetone to yield the corresponding iodo derivative which was immediately reacted with the appropriate sulfonamide derivative in the presence of DBU to give the corresponding crude products. These were purified using flash chromatography. The physical and spectral data for compounds **1**–**17** are listed in Table 1.

Biochemical studies

The apparent second order inactivation rate constants ($k_{inact}/K_I\,M^{-1}\,s^{-1}$) for compounds **1**–**17** were determined in duplicate using the progress curve method[24] and are listed in Table 2. Typical progress curves for the hydrolysis of MeOSuc-AAPV pNA by HLE in the presence of inhibitor **1** are shown in Fig. 1. The release of *p*-nitroaniline was monitored at 410 nm. Data analysis was carried out as described previously.[15–20]

The loss of enzymatic activity in the presence of inhibitor **9** as a function of time was determined by the incubation method[25] by removing aliquots and assaying for enzymatic activity (Fig. 2).

Molecular modeling

Modeling studies were performed using the Tripos force field of SYBYL 6.5 (Tripos Associates, St. Louis, MO) and a Silicon Graphics O2 workstation. The crystal structure of HLE bound to the Turkey Ovomucoid Inhibitor Third Domain (TOMI)[26] (Brookhaven, 1PPF) was used in the modeling studies by superimposing the energy-minimized inhibitor on the -P_2-P_1-P_1'-P_2'- (-Thr-Leu-Glu-Tyr-)[27] segment of TOMI with the α-carbon of Leu-18 (P_1 residue) of TOMI. A shaded surface for

Table 1. Physical constants and spectral data of inhibitors **1**–**17**

Compd[a]	^{1}H NMR (δ)	MF (anal.)
1	0.98(m,12H); 1.78(m,1H); 1.90–2.05(m,3H); 2.90(s,3H); 3.40(s,3H); 3.84(t,1H); 4.10(m,2H); 5.70(s,2H)	$C_{14}H_{12}N_3O_7S_2$ (C,H,N)
2	0.98(m,9H); 1.45(dd,1H); 1.75(m,4H); 1.95(m,1H); 2.90(s,3H); 3.40(s,3H); 3.88(t,1H); 4.30(m,2H); 5.68(s,2H)	$C_{14}H_{27}N_3O_7S_2$ (C,H,N)
3	0.95(m,6H); 1.74(m,2H); 1.88(m,1H); 2.82(s,3H); 3.38(s,3H); 3.72(t,1H); 5.30(dd,2H); 5.65(s,2H); 7.40(m,5H)	$C_{17}H_{25}N_3O_7S_2$ (C,H,N)
4	0.98(dd,2H); 1.75(m,2H); 1.92(m,1H); 2.85(s,3H); 3.80(s,3H); 5.80(dd,2H); 7.52–8.05(m,5H)	$C_{16}H_{23}N_3O_7S_2$ (C,H,N)
5	0.88(t,3H); 0.98(t,6H); 1.28(m,2H); 1.60(m,2H); 1.76(m,2H); 1.92(m,1H); 2.85(s,3H); 3.80(t,1H); 4.15(t,2H); 5.80(dd,2H);7.52–7.64(m,3H); 8.02(d,2H)	$C_{19}H_{29}N_3O_7S_2$ (C,H,N)
6	0.95(dd,6H); 1.70(m,2H); 1.85(m,1H); 2.85(s,3H); 3.45(s,3H); 3.75(t,1H); 5.70(s,2H); 7.50–7.65(m,5H)	$C_{16}H_{23}N_3O_6S_2$ (C,H,N)
7	0.98(dd,6H); 1.75(m,2H); 1.90(m,1H); 2.85(s,3H); 3.22(s,3H); 3.82(t,1H); 4.12(s,2H); 5.65(s,2H); 7.30(m,5H)	$C_{17}H_{25}N_3O_6S_2$ (C,H,N)
8	0.95(m,6H); 1.75(m,2H); 1.90(m,1H); 2.44(s,3H); 2.84(s,3H); 3.80(t,1H); 5.70(s,2H); 6.68(d,2H); 7.68(d,2H)	$C_{16}H_{24}N_3O_6S_2$ (C,H,N)
9	0.97(dd,6H); 1.44(s,9H); 1.78(t,2H); 1.90(m,1H); 2.89(s,3H); 3.44(s,3H); 3.89(t,1H); 4.32(t,2H); 5.39(t,1H); 5.67(s,2H)	$C_{16}H_{30}N_4O_8S_2$ (C,H,N)
10	0.70–0.8(dd,6H); 1.60(m,1H); 1.70(m,2H); 1.45(s,9H); 3.45(s,3H); 3.95(t,1H); 4.35(d,2H); 4.25–4.5(dd,2H); 5.10(s,2H); 5.3(s,1H); 5.7(d,2H); 7.35(s,5H)	$C_{22}H_{34}N_4O_8S_2$ (C,H,N)
11	0.96(dd,6H); 1.70–1.98(m,3H); 2.86(s,3H); 3.42(s,3H); 3.87(t,1H); 4.40(t,2H); 5.10(s,2H); 5.64(s br, 1H); 5.65(s,2H); 7.34(s,5H)	$C_{19}H_{28}N_4O_6S_2$ (C,H,N)
12	0.70–0.8(dd,6H); 1.60(m,1H); 1.75(m,2H); 3.45(s,3H); 3.95(t,1H); 4.4(d,2H); 4.25–4.55(dd,2H); 5.15(s,2H); 5.55(t,1H); 5.7(d,2H); 7.4(m,10H)	$C_{25}H_{32}N_4O_8S_2$ (C,H,N)
13	1.00(dd,6H); 1.75(m,2H); 1.90(m,1H); 2.90(s,3H); 4.10(t,1H); 4.45(m,2H); 5.10(s,2H); 5.80(s,2H); 6.70(br t,1H); 7.35–8.10(m,10H)	$C_{24}H_{30}N_4O_6S_2$ (C,H,N)
14	0.65–0.8(dd,6H); 1.30(t,1H); 1.70(m,3H); 3.40(s,3H); 3.92(t,1H); 4.10(t,2H); 4.22(m,2H); 4.26–4.55(dd,2H); 5.7(dd,2H); 7.35(m,5H)	$C_{20}H_{30}N_5O_8S_2$ (C,H,N)
15	3.10(m,2H); 3.78(s,3H); 4.0–4.25(dd,2H); 4.05(m,1H); 5.80(dd,2H); 7.0–8.05(m,15H)	$C_{25}H_{31}N_3O_7S_2$ (C,H,N)
16	0.90(t,3H); 1.30(m,2H); 1.60(m,2H); 4.05(m,1H); 3.02–3.12(dd,2H); 3.98–4.25(dd,2H); 4.15(t,2H); 5.80(dd,2H); 7.0–7.65(m,13H); 8.02(d,2H)	$C_{28}H_{31}N_3O_7S_2$ (C,H,N)
17	0.95(t,3H); 1.40(m,2H); 1.70(m,2H); 3.05(t,2H); 3.25(s,3H); 4.12(m,2H); 4.28(m,2H); 5.65(s,2H); 4.05–4.35(dd,2H); 7.10–7.30(m,10H)	$C_{23}H_{29}N_3O_7S_2$ (C,H,N)

[a]All compounds are oils and have the L configuration.

Table 2. Inhibitory activity of derivatives of (I) toward human leukocyte elastase, cathepsin G and proteinase 3

Compd	P_1	R_2	R_3	R_4	k_{inact}/K_I $M^{-1}s^{-1}$ HLE	PR3	Cat G
1	Isobutyl	Methyl	Isobutyloxy	Methyl	11,900	1200	—[a]
2	Isobutyl	Methyl	*n*-butyloxy	Methyl	40,500	3400	—
3	Isobutyl	Methyl	Benzyloxy	Methyl	22,000	3000	—
4	Isobutyl	Methyl	Methoxy	Phenyl	51,100	6100	—
5	Isobutyl	Methyl	*n*-butyloxy	Phenyl	70,500	6400	—
6	Isobutyl	Benzyl	Phenyl	Methyl	71,000	16,000	—
7	Isobutyl	Methyl	Benzyl	Methyl	28,300	10,100	200
8	Isobutyl	Methyl	Methyl	(*p*-NH_2)phenyl	9900	3,200	—
9	Isobutyl	Methyl	CH_2NHBoc	Methyl	140,500	27,500	—
10	Isobutyl	Benzyl	CH_2NHBoc	Methyl	148,900	2400	—
11	Isobutyl	Methyl	CH_2NHCbz	Methyl	229,400	27,400	60
12	Isobutyl	Benzyl	CH_2NHCbz	Methyl	134,000	30,100	70
13	Isobutyl	Methyl	CH_2NHCbz	Phenyl	92,700	29,200	[b]
14	Isobutyl	Benzyl	$NHCH_2COOEt$	Methyl	14,100	5200	70
15	Benzyl	Benzyl	Methoxy	Phenyl	—	[b]	90
16	Benzyl	Benzyl	*n*-butyloxy	Phenyl	—	[b]	80
17	Benzyl	Benzyl	*n*-butyloxy	Methyl	1970	[b]	5400

[a]Not determined.
[b]Compounds designated as inactive showed less than 50% inhibition when incubated with the enzyme at an inhibitor to enzyme ratio of 250 for 10 minutes.

HLE was then generated following the removal of TOMI and water molecules (Fig. 3).

Discussion

The majority of covalent and non-covalent inhibitors of enzymes with extended binding sites such as, for example, proteases, have generally been designed to exploit favorable binding interactions *only* with the S subsites of a target enzyme.[14,28] Furthermore, since the *primary* specificity of a serine or cysteine protease is ordinarily determined by the nature of the P_1 residue, discrimination between neutral, basic and acidic proteases has been achieved by incorporating an appropriate P_1 residue, or equivalent, in the structure of an inhibitor. Thus, inhibitors having a *neutral* (hydrophobic) P_1 residue are intended to inhibit neutral serine or cysteine proteases such as chymotrypsin, elastase, chymase, PSA, cathepsin G, etc.; those with a *basic* P_1 residue inhibit basic proteases such as thrombin, factor Xa, urokinase-type plasminogen activator, kallikrein, plasmin, tryptase, granzyme A, cathepsin K, gingipain, etc.; and those with an *acidic* P_1 residue inhibit acidic proteases (granzyme B, caspases). Recently, we have demonstrated that inhibitors based on the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold to which an appropriate recognition element P_1 has been appended exhibit absolute or near absolute enzyme selectivity between neutral, basic and acidic proteases.[18–20]

The design of inhibitors that exhibit high enzyme selectivity among members of the *same* subclass has been problematic, since members of a given subclass show a preference for the same P_1 residue. Nevertheless, inspection of the available X-ray crystal structures of (chymo)trypsin-like proteases reveals the existence of subtle differences in their S′ and S subsites. Exploitation of these differences could, in principle, yield inhibitors that display high enzyme selectivity.

Attaining maximal enzyme selectivity is a problem of paramount importance in drug design, particularly when considering the inhibition of proteases. For example, urokinase-type plasminogen activator (uPA) is a serine protease that shows a strong preference for an arginine as the P_1 residue. This enzyme appears to play a pivotal role in cancer metastasis, consequently

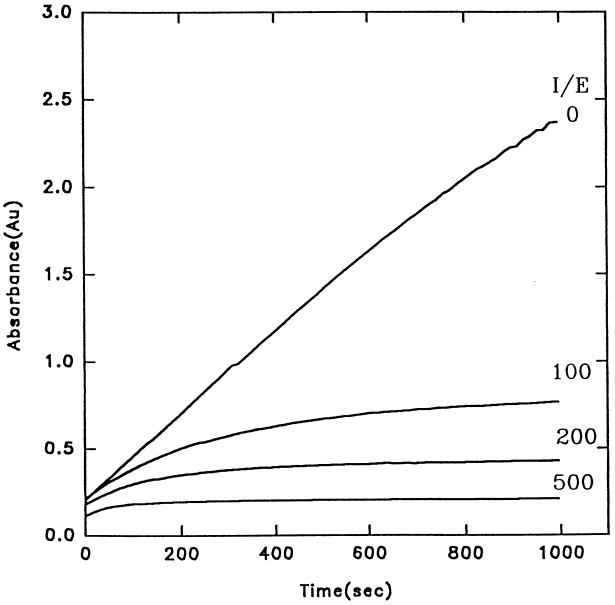


Figure 1. Progress curves for the inhibition of human leukocyte elastase (HLE) by compound **1**. Absorbance was recorded at 410 nm for reaction solutions containing HLE (21.9 nM), MeOSuc-AAPV pNA (1 mM) at the indicated inhibitor to enzyme ratios in 0.1 M HEPES buffer containing 0.5 M NaCl, pH 7.25 and 3.6% dimethyl sulfoxide. The temperature was maintained at 25 °C, and the reactions were initiated by the addition of enzyme.

inhibitors that display high inhibitory activity toward uPA and low or no activity toward related proteases (thrombin, factor Xa, plasmin) are of potential value as anticancer agents.[29] Clearly, uPA inhibitors with low enzyme selectivity would result in unacceptable toxicity since they could potentially disrupt the blood coagulation cascade.

We hypothesized that the rigorous spatial control afforded by the 1,2,5-thiadiazolidin-3-one 1,1 dioxide template, and the fact that the template makes possible the exploitation of binding interactions with both the S and S′ subsites, might make possible the attainment of optimal enzyme selectivity. In an attempt to achieve this aim, we have pursued a strategy that explores the use of functionalized sulfonamides as a novel design element which, when coupled to the aforementioned heterocyclic template, might yield inhibitors having the desired characteristics. In conducting these preliminary studies, P_1 was chosen to be either isobutyl or benzyl, in accordance with the known substrate specificity of HLE and Cat G, respectively. The use of R_2 = methyl was based on earlier observations that showed that inhibitors with a methyl group at R_2 yield stable acyl enzymes.[15]

Incubation of compound **9**, for example, with human leukocyte elastase led to rapid and essentially irreversible inactivation of the enzyme. The inactivation of the enzyme by **9** was time-dependent and very efficient, requiring only three equiv of **9** for total inactivation of the enzyme (Fig. 2). Furthermore, the putative acyl enzyme formed exhibits high stability, as evidenced by the partial and slow regain of enzymatic activity over a 24-h period.

Two significant and general deductions can be made by inspecting the results shown in Table 2. First, sulfonamide derivatives based on the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold are potent inhibitors of HLE and PR 3. Secondly, a high level of structural diversity can be introduced into the sulfonamide moiety without compromising inhibitory activity.

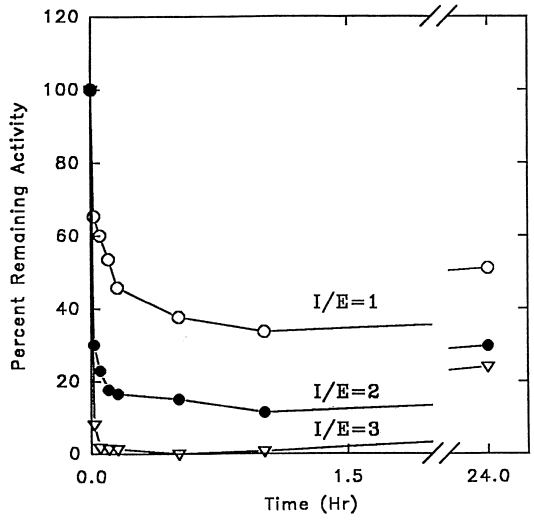


Figure 2. Percent remaining activity versus time plot obtained by incubating inhibitor **9** with human leukocyte elastase (138 nM) at different inhibitor to enzyme ratios in 0.1 M HEPES buffer containing 0.5 M NaCl, pH 7.25, and 1% DMSO. Aliquots were withdrawn at different time intervals and assayed for enzymatic activity using MeOSuc-AAPV pNA by monitoring the absorbance at 410 nm.

The results in Table 2 also show that the most potent compounds are those in which the sulfonamide leaving group is derived from a protected glycine (**9**–**13**). Replacement of glycine by other amino acids is anticipated to enhance inhibitory activity, since the side chain of the amino acid would make additional favorable contacts with the S_2' subsite (Fig. 4). Indeed, compound **18** (Fig. 5) is a more efficient inhibitor of HLE than compound **14** (Table 2) (k_{inact}/K_I 35,000 versus 14,100 $M^{-1} s^{-1}$). Figure 3 illustrates the binding of energy-minimized inhibitor **18** to the active site of HLE. The isobutyl and *N*-benzyl groups occupy the hydrophobic S_1 and S_2 pockets, while the sulfonamide moiety orients itself toward the S′ pockets. Thus, the benzyl group of the amino acid is likely involved in specific hydrophobic interactions with Phe-41 and/or Phe-192. Definitive evidence regarding the precise mode of binding, and the network of hydrophobic and hydrogen

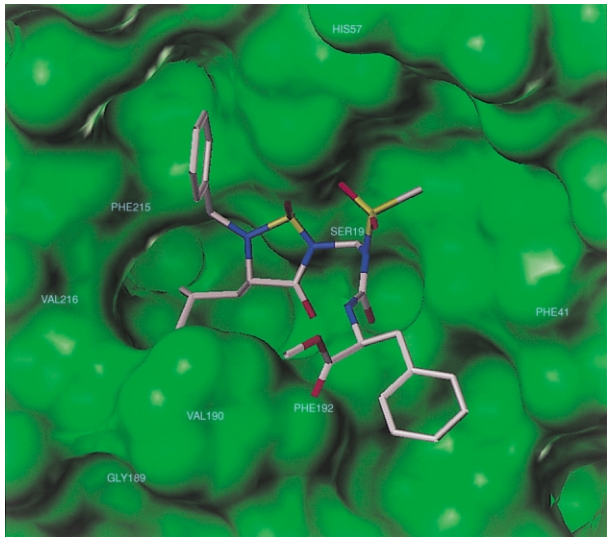


Figure 3. Energy-minimized inhibitor **18** docked to the active site of HLE with the isobutyl (P_1) and benzyl groups (P_2) occupying the S_1 and S_2 subsites, respectively. The functionalized sulfonamide segment is oriented toward the S′ subsites, with the benzyl group of (L) Phe sandwiched between Phe 41 and Phe 192 and occupying the S_2' subsite.

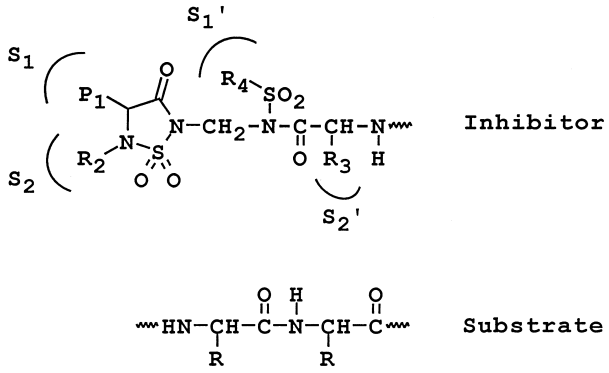


Figure 4. Postulated binding of compound (I) to the active site and interactions with the S′ subsites.

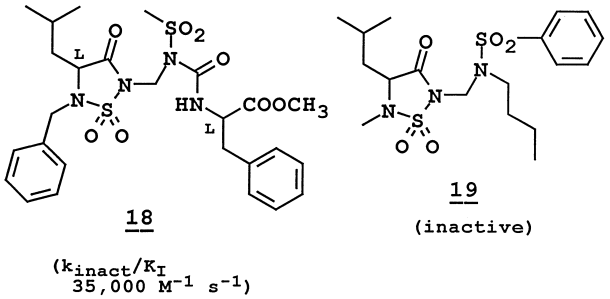


Figure 5. Structures and inhibitory activity of compounds **18** and **19**.

bonding interactions involved, will have to await the determination of the X-ray crystal structure of the enzyme-inhibitor complex.

In principle, potency, enzyme selectivity, and pharmacokinetics can be readily optimized by deblocking the amino acid and derivatizing the resulting product further. Interestingly, the replacement of a methyl group with phenyl (compound **13**) results in potent inhibition of HLE and PR 3 but not Cat G (vide infra). Equally noteworthy is the observation that compound **14** is fairly active toward HLE and PR 3. Since the precursor sulfonamide derivatives for making compounds like **14** are made by reacting a sulfonamide with an isocyanate, the use of readily available amino acid-derived isocyanates[30,31] greatly enhances the potential of these compounds. The lower potency of compound **14** versus compounds **9**–**13** is partly attributed to the lower leaving group ability of the sulfonamide group in **14**. It should be noted that (a) the pK_a of functionalized sulfonamides can be modulated by varying the nature of R_3 and R_4 and, (b) the pK_a of the leaving group has a profound effect on inhibitory activity. Indeed, *N*-alkyl substituted sulfonamides (for example, compound **19** in Fig. 5) appended to the heterocyclic scaffold are devoid of inhibitory activity.

In all cases, the synthesized compounds inhibited both HLE and PR 3, reflecting the similarity of their active sites.[32] As anticipated, these compounds were more effective inhibitors of HLE than PR 3, since the P_1 residue was chosen to be optimal for HLE than PR 3. The active site of the latter is more constricted, showing a greater preference for small linear alkyl chains(ethyl) *n*-propyl).[33–35]

The inhibitory activity of compounds **2**–**5** (R_3 = alkoxy) toward HLE and PR 3 was significant but no dramatic variations in potency were observed. The analogous Cat G inhibitors were substantially weaker, however more secure inferences regarding the inhibition of Cat G by this class of compounds will have to await the results of more extensive SAR studies.

This study has conclusively established for the first time that functionalized sulfonamides constitute a powerful design element that (a) when coupled to the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold yields potent, time-dependent inhibitors of HLE and PR 3; (b) possesses low intrinsic chemical reactivity and, (c) offers considerable flexibility with respect to the generation of structurally-diverse single compounds or libraries. Exploratory studies focusing on the utility and scope of functionalized sulfonamides in drug design are currently in progress and the results will be reported in due course.

Experimental

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra of the synthesized compounds were recorded on a Varian XL-300 NMR spectrometer. A Hewlett-Packard diode array UV/Vis spectrophotometer was used in the enzyme assays and inhibition studies. Human leukocyte elastase was purchased from Elastin Products Company, Owensville, MO. Human leukocyte cathepsin G and proteinase 3 were purchased from Athens Research and Technology Co., Athens, GA. Methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide and methoxysuccinyl Ala-Ala-Pro-Phe *p*-nitroanilide were purchased from Sigma Chemical Co., St. Louis.

General procedure for the synthesis of compounds 1–17

A solution of chloromethyl compound (2 mmol) in dry acetone (10 mL) kept under nitrogen was treated with sodium iodide (4 mmol), and the resulting mixture was stirred at room temperature overnight. The precipitate (NaCl) was filtered off and the solvent evaporated off, leaving the corresponding iodo compound. This was dissolved in dry methylene chloride (10 mL) and treated with the appropriate sulfonamide (2 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (2 mmol). The reaction mixture was stirred at room temperature overnight under a nitrogen atmosphere. Removal of the solvent left a crude product which was taken up in ethyl acetate (60 mL) and washed with water (20 mL), 5% sodium bicarbonate (2×15 mL) and brine (20 mL). The organic layer was dried and evaporated, leaving a crude product which was purified by flash chromatography using silica gel (hexane/ether gradient).

Acknowledgements

This work was supported by grants from the National Institutes of Health (HL 57788) and Supergen, Inc.

References

1. Dhami, R.; Gilks, B.; Xie, C.; Zay, K.; Wright, J. L.; Churg, A. *Am. J. Respir. Cell Mol. Biol.* **2000**, *22*, 244.
2. Ofulue, A. F.; Ko, M. *Am. J. Physiol.* **1999**, *277*, L97.
3. Ohnishi, K.; Takagi, M.; Kurokawa, Y.; Satomi, S.; Konttinen, Y. T. *Lab. Invest.* **1998**, *78*, 1077.
4. ; Stockley, R. A. *Am. J. Respir. Crit. Care Med.* **1994**, *150*, S109.
5. Jochum, M.; Gippner-Steppert, C.; Machleidt, W.; Fritz, H. *Am. J. Respir. Crit. Care Med.* **1994**, *150*, S123.
6. Barnes, P. J.; Am, J. *Respir. Crit. Care Med.* **1999**, *160*, S72.

7. Witko-Sarsat, V.; Halbwachs-Mecarelli, L.; Schuster, A.; Nusbaum, P.; Ueki, I.; Canteloup, S.; Lenoir, G.; Descamps-Latscha, B.; Nadel, J. A. *Am. J. Respir. Cell Mol. Biol.* **1999**, *20*, 729.
8. Schuster, A.; Fahy, J. V.; Nadel, J. A. *Eur. Respir. J.* **1995**, *8*, 10.
9. Travis, J.; Pike, R.; Inamura, T.; Potempa, J. *J. Am. Respir. Crit. Care Med.* **1994**, *150*, S123.
10. Wiedow, O.; Wiese, F.; Christophers, E. *Arch. Dermatol. Res.* **1995**, *287*, 632.
11. ; Stockley, R. A. *Am. J. Respir. Crit. Care Med.* **1999**, *160*, S49.
12. Vignola, A. M.; Riccobono, L.; Mirabella, A.; Profita, M.; Chanez, P.; Bellia, V.; Mautino, G.; D'Accardi, P.; Bousquet, J.; Bonsignore, G. *Am. J. Respir. Crit. Care Med.* **1988**, *158*, 1945.
13. Anderson, G. P.; Shinagawa, K. *Curr. Op. Antiinfl. Immun. Invest. Drugs* **1999**, *1*, 29.
14. Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305.
15. Groutas, W. C.; Kuang, R.; Venkataraman, R.; Epp, J. B.; Ruan, S.; Prakash, O. *Biochemistry* **1997**, *36*, 4739.
16. Groutas, W. C.; Kuang, R.; Ruan, S.; Epp, J. B.; Venkataraman, R.; Truong, T. M. *Bioorg. Med. Chem.* **1998**, *6*, 661.
17. Kuang, R.; Venkataraman, R.; Ruan, S.; Groutas, W. C. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 539.
18. Kuang, R.; Epp, J. B.; Ruan, S.; Yu, H.; Huang, P.; He, S.; Tu, J.; Schechter, N. M.; Turbov, J.; Froelich, C. J.; Groutas, W. C. *J. Am. Chem. Soc.* **1999**, *121*, 8128.
19. Groutas, W. C.; Schechter, N. M.; He, S.; Yu, H.; Huang, P.; Tu, J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2199.
20. Kuang, R.; Epp, J. B.; Ruan, S.; Chong, L. S.; Venkataraman, R.; Tu, J.; He, S.; Truong, T. M.; Groutas, W. C. *Bioorg. Med. Chem.* **2000**, *8*, 1005.
21. Drummond, J. T.; Johnson, G. *Tetrahedron Lett.* **1988**, *29*, 1653.
22. Neustadt, B. R. *Tetrahedron Lett.* **1994**, *35*, 379.
23. Majer, P.; Randad, R. S. *J. Org. Chem.* **1994**, *59*, 1937.
24. Morrison, J. F.; Walsh, C. T. *Adv. Enzymol.* **1989**, *61*, 201.
25. Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *12*, 2940.
26. Bode, W.; Wei, A.-Z.; Huber, R.; Meyer, E. F.; Travis, J.; Neumann, S. *EMBO J.* **1986**, *5*, 2453.
27. Schechter, I.; Berger, A. *Biochem. Biophys. Res. Comm.* **1967**, *27*, 157.
28. Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
29. Sperl, S.; Jacob, U.; de Prada, A. A.; Strurzebecher, J.; Wilhelm, O. G.; Bode, W.; Magdolen, V.; Huber, R.; Moroder, L. *Proc. Nat. Acad. Sci. U.S.A.* **2000**, *97*, 5113.
30. Eckert, H.; Forster, B. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 894.
31. Knolker, H.-J.; Braxmeir, T. *Synlett* **1997**, 925.
32. Fujinaga, M.; Chernaia, M. M.; Halenbeck, R.; Koths, K.; James, M. N. G. *J. Mol. Biol.* **1996**, *261*, 267.
33. Groutas, W. C.; Hoidal, J. R.; Brubaker, M. J.; Stanga, M. A.; Venkataraman, R.; Gray, B. H.; Rao, N. V. *J. Med. Chem.* **1990**, *33*, 1085.
34. Brubaker, M. J.; Groutas, W. C.; Hoidal, J. R.; Rao, N. V. *Biochem. Biophys. Res. Comm.* **1992**, *188*, 1318.
35. Kam, C. M.; Kerrigan, J. E.; Dolman, K. M.; Goldschmeding, R.; Von dem Borne, A. E.; Powers, J. C. *FEBS Lett.* **1992**, *297*, 119.