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Potent Inhibition of Serine Proteases by Heterocyclic Sulfide Derivatives of 1,2,5-Thiadiazolidin-3-one 1,1 Dioxide

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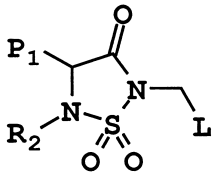
Abstract—The existence of subtle differences in the S_n' subsites of closely-related (chymo)trypsin-like serine proteases, and the fact that the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold docks to the active site of (chymo)trypsin-like enzymes in a substrate-like fashion, suggested that the introduction of recognition elements that can potentially interact with the S_n' subsites of these proteases might provide an effective means for optimizing enzyme potency and selectivity. Accordingly, a series of heterocyclic sulfide derivatives based on the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold (I) was synthesized and the inhibitory activity and selectivity of these compounds toward human leukocyte elastase (HLE), proteinase 3 (PR 3) and cathepsin G (Cat G) were then determined. Compounds with P_1 = isobutyl were found to be potent, time-dependent inhibitors of HLE and, to a lesser extent PR 3, while those with P_1 = benzyl inactivated Cat G rapidly and irreversibly. This study has demonstrated that 1,2,5-thiadiazolidin-3-one 1,1 dioxide-based heterocyclic sulfides are effective inhibitors of (chymo)trypsin-like serine proteases.

Introduction

It is generally accepted that the existence of a protease–antiprotease imbalance is associated with various pathological states, including cystic fibrosis, pulmonary emphysema, chronic bronchitis, arthritis and others.[1–5] Proteases shown to play a major role in these ailments include the serine endopeptidases neutrophil elastase, cathepsin G, proteinase 3 and macrophage-derived metalloproteases.[6] The activity of these enzymes can, in principle, be regulated via the administration of known endogenous protein inhibitors of these enzymes such as, for example, alpha-1-proteinase inhibitor, secretory leukocyte protease inhibitor, human monocyte/neutrophil elastase inhibitor or TIMPs (tissue inhibitors of metalloproteases). Alternatively, because (a) the efficacy of orally-administered peptide and protein drugs is often compromised by their poor absorption, low metabolic stability and rapid excretion and, (b) the fact that cell surface-bound elastase and cathepsin G are resistant to inhibition by protein inhibitors but are inhibited by low molecular weight inhibitors,[7] the use of low molecular weight inhibitors to redress the protease-antiprotease imbalance is a promising avenue of investigation that may lead to the emergence of useful therapeutic agents for chronic obstructive lung diseases and other inflammatory ailments.[8–10]

We have recently described the structure-based design of a novel peptidomimetic template and have demonstrated that the template can serve as a general scaffold for the design of potent and selective inhibitors, including libraries of inhibitors, of serine proteases having a (chymo)trypsin-like fold.[11–15] We wish to describe herein the synthesis and in vitro evaluation of a series of heterocyclic sulfides appended to the 1,2,5-thiadiazolidin-3-one 1,1 dioxide (I) scaffold as time-dependent inhibitors of human leukocyte elastase (HLE), proteinase 3 (PR 3) and cathepsin G (Cat G).



(I)

Results

Chemistry

Compounds **1**–**17** were synthesized by adding triethylamine (2.2 mmol) to a solution of (L) 2-chloromethyl-4-

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

Compounds	MP (°C)	^{1}H NMR(δ)	MF (anal.)
1	Oil	0.98 (dd, 6H); 1.80 (m, 2H); 1.90 (m, 1H); 2.88 (s, 3H); 3.88 (t, 1H); 5.52 (d, 2H); 7.48 (m, 3H); 8.08 (d, 2H).	$C_{16}H_{20}N_4O_4S_2$ (C,H,N)
2	97–99	0.65–0.80 (dd, 6H); 1.58 (m, 2H); 1.70(m. 1H); 3.90 (t, 1H); 4.24-4.50 (dd, 2H); 5.52 (dd, 2H); 7.35–8.10 (m, 10H).	$C_{21}H_{24}N_3O_4S_2$ (C,H,N)
3	Oil	0.95 (dd, 6H); 1.75 (m, 2H); 1.90 (m, 1H); 2.84 (S, 3H); 3.82 (t, 1H); 5.60 (d, 2H); 7.32-7.95 (m, 9H).	$C_{15}H_{19}N_3O_3S_3$ (C,H,N)
4	Oil	0.65–0.85 (dd, 6H); 1.52 (m, 2H); 1.80 (m 1H); 3.92 (t, 1H); 4.28–4.53 (dd, 2H); 5.62 (dd, 2H); 7.3–7.96 (m, 9H).	$C_{21}H_{23}N_3O_3S_3$ (C,H,N)
5	72–74	0.95 (dd, 6H); 1.78 (m, 2H); 1.88 (m, 1H); 2.85 (s, 3H); 3.82 (t, 1H); 5.50 (dd, 2H); 7.30–7.68 (m, 4H).	$C_{13}H_{19}N_3O_4S_2$ (C,H,N)
6	83–84	0.68–0.78 (dd, 6H); 1.58 (m, 2H); 1.70 (m, 1H); 3.88 (t, 1H); 4.25–5.52 (dd, 2H); 5.50 (dd, 2H); 7.28–7.65 (m, 9H).	$C_{21}H_{23}N_3O_4S_2$
7	Oil	0.95 (dd, 6H); 1.75 (m, 2H); 1.88 (m, 1H); 2.85 (s, 3H); 3.83 (t, 1H); 5.38 (d, 2H); 7.35–7.65 (m, 10H).	$C_{23}H_{25}N_3O_4S_2$ (C,H,N)
8	Oil	0.60–0.75 (dd, 6H); 1.50 (m, 2H); 1.70 (m, 1H); 3.85 (t, 1H); 4.24–4.50 (dd, 2H); 5.35 (dd, 2H); 7.3–7.65 (m, 10H).	$C_{29}H_{29}O_4S_2$ (C,H,N)
9	Oil	0.95 (dd, 6H); 1.78 (m, 1H); 1.90 (m, 2H); 2.88 (s, 3H)); 3.85 (t, 1H); 5.35 (s, 2H); 7.50 (m, 3H); 8.05 (d, 2H).	$C_{16}H_{20}N_4O_4S_2$ (C,H,N)
10	90–91.5	0.64–0.78 (dd, 6H); 1.55 (m, 2H); 1.70 (m, 1H); 3.90 (t, 1H); 4.25–4.50 (dd, 2H); 5.35 (dd, 2H); 7.35-8.05 (m, 10H).	$C_{22}H_{24}N_4O_4S_2$ (C,H,N)
11	Oil	0.95 (dd, 6H); 1.8 (m, 2H); 1.909m, 1H); 2.87 (s, 3H); 3.85 (t, 1H); 5.52 (dd, 2H); 7.3–7.85 (m, 8H).	$C_{21}H_{23}N_3O_4S_2$ (C,H,N)
12	93–94.5	0.65–0.80 (dd, 6H); 1.58 (m, 2H); 1.70 (m, 1H); 3.90 (t, 1H); 4.25–4.50 (dd, 2H); 5.52 (dd, 2H); 7.35–7.80 (m, 13H).	$C_{25}H_{27}N_3O_4S_2$
13	Oil	2.64 (S, 3H); 3.10–3.25 (dd, 2H); 4.05 (m, 1H); 5.30 (S, 2H); 7.20–8.10 (m, 10H).	$C_{19}H_{18}N_4O_4S_2$ (C,H,N)
14	115–117	3.05 (m, 2H); 4.02–4.32 (dd, 2H); 4.10 (m, 1H); 5.32 (dd, 2H); 7.05–8.05 (m, 10H).	$C_{25}H_{27}N_4O_4S_2$ (C,H,N)
15	Oil	2.64 (S, 3H); 3.10–3.35 (dd, 2H); 4.10 (m, 1H); 5.32 (dd, 2H); 7.05–8.05 (m, 15H).	$C_{25}H_{22}N_4O_4S_2$ (C,H,N)
16	14.2–14.5	2.62 (s, 2H); 3.05–3.31 (dd, 2H); 4.05 (m, 1H); 5.45 (dd, 2H); 7.20–7.65 (m, 14H).	$C_{24}H_{21}N_3O_4S_2$ (C,H,N)
17	Oil	3.05 (m, 2H); 4.0–4.32 (dd, 2H); 4.05 (t, 1H);5.42 (d, 2H); 6.80 (d, 2H); 7.0–7.25 (m, 10H); 7.70 (d, 2H).	$C_{24}H_{22}N_2O_3S_3$ (C,H,N)

isobutyl-5-methyl(or benzyl)-1,2,5-thiadiazolidin-3-one 1,1 dioxide[11] (2 mmol) or (L) 4-benzyl-2-chloromethyl-5-methyl (or benzyl)-1,2,5-thiadiazolidin-3-one 1,1 dioxide[11] (2 mmol) and the appropriate heterocyclic thiol (2.2 mmol) in dry acetonitrile (6 mL) and either stirred at room temperature overnight or refluxed for 2 h. Work up yielded the crude sulfides which were purified using flash chromatography. The physical properties and spectral data of the synthesized compounds are listed in Table 1. The structures of the heterocyclic thiols used in the synthesis of compounds **1–17** are listed in Table 3. A representative synthesis is described in Experimental.

Biochemical studies

The rates of inactivation of HLE, Cat G and PR 3 by each compound were determined by the progress curve method.[11,16–17] Typical progress curves using a representative heterocyclic sulfide are illustrated in Fig. 1. The apparent second order rate constants (k_{inact}/K_I M^{-1} s^{-1}), the magnitude of which serves as an index of inhibitory potency, were determined in duplicate and are listed in Table 2. The reproducibility of these values was within plus or minus 10%.

Molecular modeling

Modeling studies were performed using the Tripos force field of SYBYL 6.5 (Tripos Associates, St. Louis, MO) using a Silicon Graphics O2 workstation. The crystal structure of HLE bound to the Turkey Ovomucoid Inhibitor Third Domain[18] (Brookhaven, 1PPF) was used in the modeling studies by superimposing the energy-minimized inhibitor on the -Thr-Leu-Glu-Tyr- (-P_2-P_1-P_1'-P_2'-)[19] segment of TOMI with the alpha-carbon and carbonyl group of the inhibitor overlapping the corresponding alpha-carbon of Leu-18 (P_1 residue) of TOMI. TOMI and water molecules were deleted and a shaded surface for HLE was generated.

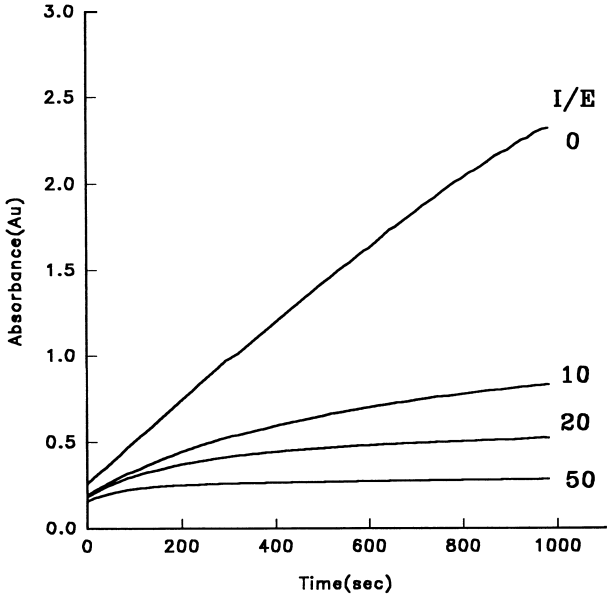


Figure 1. Progress curves for the inhibition of human leukocyte elastase (HLE) by compound **6.** Absorbance was recorded at 410 nm for reaction solutions containing HLE (21.9 nM), MeOSuc-AAPV-pNA (1 mM) and the indicated concentrations of inhibitor **6** in 0.1 M HEPES buffer containing 0.5 M NaCl, pH 7.25 and 3.6% DMSO. The temperature was maintained at 25 °C, and the reactions were initiated by the addition of enzyme.

Discussion

We have recently shown that the 1,2,5-thiadiazolidin-3-one 1,1 dioxide platform docks to the active site of (chymo)trypsin-like serine proteases in a predictable and substrate-like fashion,[11–15] namely, with the P_1 group accommodated at the primary specificity pocket (S_1)[19] and the R_2 and L groups oriented toward the S_2 and S_n' subsites, respectively. While the S_n subsites of (chymo)trypsin-like serine proteases have been explored and potential binding interactions with those subsites exploited extensively in inhibitor design, studies focusing on probing the S_n' subsites have been limited. The primary reason for this lies with the inherent constraints and limitations associated with the structures of the inhibitors used. The fact that (a) the leaving group (L) in (I) is oriented toward the S_n' subsites, (b) a range of leaving groups can be appended to the heterocyclic template without compromising inhibitory activity and, most importantly, (c) the existence of subtle differences in the S_n' subsites of closely-related proteases, has provided the impetus behind the studies described herein, since L provides a convenient means for optimizing potency and enzyme selectivity. The successful use of saccharin-based heterocyclic sulfides in the inhibition of HLE,[20] as well as preliminary modeling studies using the HLE-TOMI complex, suggested that the incorporation of suitably-substituted heterocyclic sulfides into (I) might yield potent inhibitors of the enzyme.

Incubation of a representative heterocyclic sulfide derivative of (I) (compound **6**) with HLE led to rapid and time-dependent inactivation of the enzyme (Fig. 1). The progress curves also indicate that the interaction of (I) with HLE involves the active site. It is evident from Table 2 that heterocyclic sulfide derivatives of (I) are fairly potent inhibitors of HLE, PR 3 and Cat G. The nature of P_1 reflects the known substrate specificity of a target protease, namely, compounds intended to function as inhibitors of HLE are derived from (L) leucine (P_1 = isobutyl), while those intended to inhibit Cat G are derived from (L) Phe (P_1 = benzyl). Thus, with the exception of compound **12**, all compounds with P_1 = isobutyl (**1**–**11**) were found to inhibit HLE and to a lesser extent PR 3, but were poor inhibitors of Cat G. Likewise, compounds **13**–**17** were good inhibitors of Cat G but not HLE or PR 3. Although the active sites of HLE and PR 3 exhibit subtle differences,[21–23] nevertheless they are very similar and show a strong preference for small hydrophobic side chains as P_1. Consequently, the observation that compounds **1**–**11** inhibit both HLE and PR3 is not surprising and is consistent with previous observations.[11–14] A large body of evidence suggests that both HLE and PR 3 play an important role in lung connective tissue destruction as is observed in pulmonary emphysema, for example. Indeed, the intratracheal administration of either HLE or PR 3 to hamsters leads to extensive damage to lung connective tissue.[24,25] Thus, the availability of an agent that inhibits both enzymes may in fact be more advantageous than one that inhibits HLE or PR 3 only.

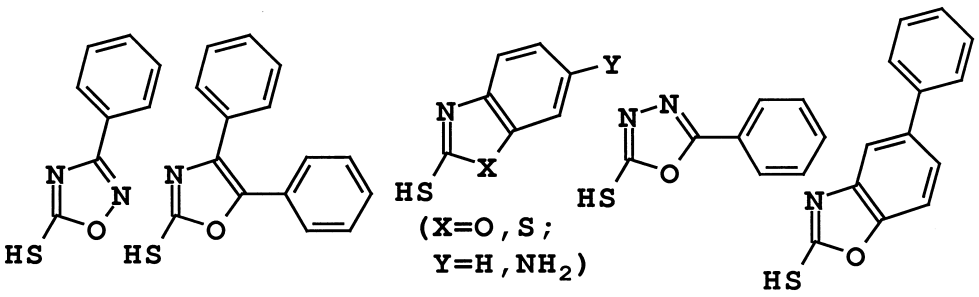
One of the major goals of the present study was to use a range of substituted heterocyclic sulfides to probe the S_1'-S_3' subsites of these enzymes. The overall character of these subsites in HLE is hydrophobic[18] and the region encompassing these subsites is rather shallow. In contrast, the Leu-143 to Arg-143 substitution in PR 3 makes the S_2' subsite of the enzyme more polar. It was anticipated that hydrophobic binding interactions with the hydrophobic heterocyclic thiols used to generate the corresponding sulfides would enhance binding. Further assurance that this would be the case was provided by modeling studies. Indeed, energy-minimized compound **1** docked to the active site of HLE has the isobutyl and benzyl groups snugly nestled into the S_1 and S_2 subsites of the enzyme and the heterocyclic sulfide leaving group oriented toward the S_n' sites (Fig. 2). The latter is flanked by Phe 41 and other hydrophobic residues. Thus, compounds **1**–**6** and **9**–**12** were all found to be fairly potent inhibitors of HLE. The reduced potency of compounds **7**–**8** arises from the inability of the hetero-

Table 2. Inhibitory activity of heterocyclic sulfides **1**–**17** toward human leukocyte elastase, proteinase 3 and cathepsin G

Compound	P_1	R_2	L	k_{inact}/k_I HLE	$M^{-1}s^{-1}$ PR3	CAT G
1	Isobutyl	Methyl	3-Phenyl-5-mercapto-1,2,4-oxadiazolyl	153,460	16,350	—[a]
2	Isobutyl	Benzyl	3-Phenyl-5-mercapto-1,2,4-oxadiazolyl	67,840	1510	—[a]
3	Isobutyl	Methyl	2-Mercaptobenzothiazolyl	67,300	5990	—[a]
4	Isobutyl	Benzyl	2-Mercaptobenzothiazolyl	22,360	1070	—[a]
5	Isobutyl	Methyl	2-Mercaptobenzoxazolyl	80,580	10,350	30
6	Isobutyl	Benzyl	2-Mercaptobenzoxazolyl	174,440	9130	60
7	Isobutyl	Methyl	4,5-Diphenyl-2-mercapto-oxazolyl	1540	1560	—[a]
8	Isobutyl	Benzyl	4,5-Diphenyl-2-mercapto-oxazolyl	560	340	—[a]
9	Isobutyl	Methyl	5-Phenyl-2-mercapto-1,2,4-oxadiazolyl	25,280	12,630	—[a]
10	Isobutyl	Benzyl	5-Phenyl-2-mercapto-1,2,4-oxadiazolyl	168,130	3590	—[a]
11	Isobutyl	Methyl	5-Phenyl-2-Mercaptobenzoxazolyl	22,630	11,100	—a
12	Isobutyl	Benzyl	5-Phenyl-2-Mercaptobenzoxazolyl	Inactive	1090	50
13	Benzyl	Methyl	5-Phenyl-2-mercapyo-1,3,4-oxadiazolyl	Inactive	—[b]	490
14	Benzyl	Benzyl	5-Phenyl-2-mercapyo-1,3,4-oxadiazolyl	Inactive	—[b]	17,460
15	Benzyl	Methyl	2-Mercaptobenzoxazolyl	—[b]	—[b]	430
16	Benzyl	Benzyl	2-Mercaptobenzoxazolyl	—[b]	—[b]	17,130
17	Benzyl	Benzyl	6-Amino-2-mercaptobenzoxazolyl	—[b]	—[b]	15,740

[a]Not determined.
[b]Less than 50% inhibition at an inhibitor to enzyme ratio of 250.

Table 3. Heterocyclic thiols



cyclic sulfide leaving group to fit into the S_n' subsites. Specifically, modeling studies with **7**–**8** clearly reveal that the phenyl rings on the oxazole ring cannot be both accommodated at the S_n' subsites. The observation that compound **12** lacks inhibitory activity is surprising and at variance with preliminary modeling studies.

In summary, we have demonstrated that heterocyclic sulfides appended to the 1,2,5-thiadiazolidin-3-one 1,1 dioxide template function as time-dependent, mechanism-based inhibitors of the serine proteases elastase, cathepsin G and proteinase 3. These results attest to the exquisite flexibility and versatility afforded by this heterocyclic platform in the design of inhibitors of (chymo) trypsin-like serine proteases.

Experimental

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. Purification of compounds by flash chromatography was carried using Merck grade silica gel (grade 60, 230400 mesh, 60 A) purchased from Aldrich Chemical Co. Thin layer chromatography performed using Analtech silica gel plates and the TLC plates were visualized by iodine vapor and/or UV light.

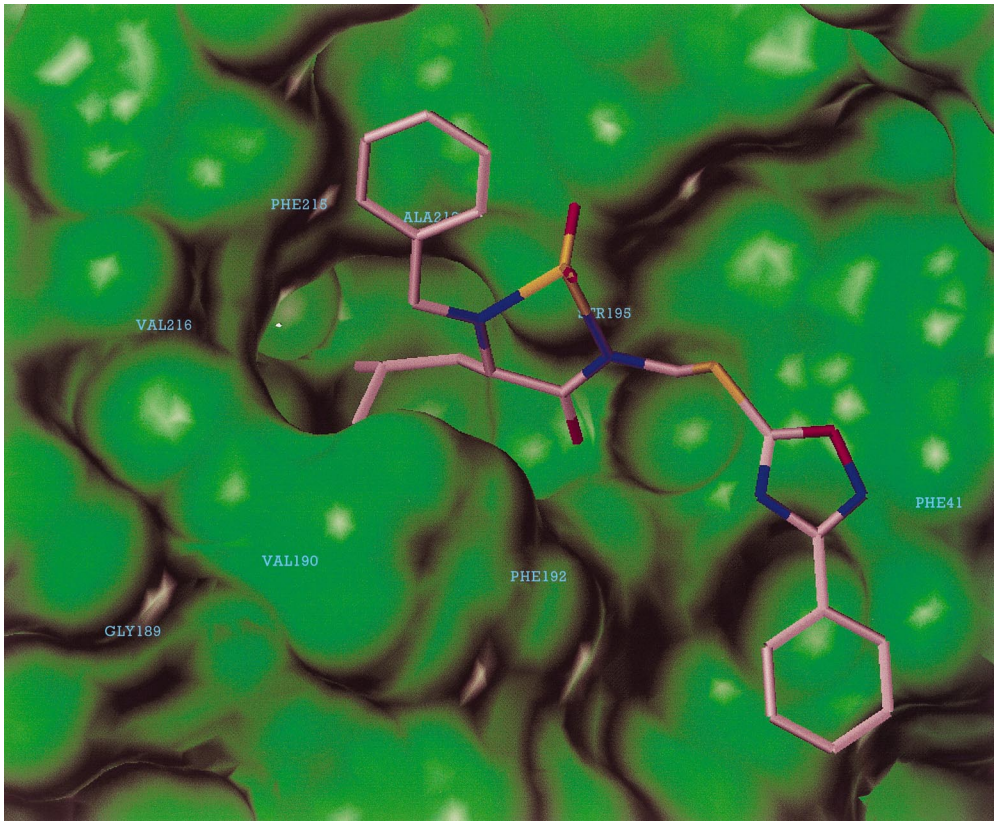


Figure 2. Energy minimized inhibitor **6** docked to the active site of HLE (shown as a shaded surface) with the isobutyl group (P_1) projecting into the S_1 pocket. The *N*-benzyl group is occupying the S_2 pocket while the 3-phenyloxadiazolyl segment is oriented toward the S_2'-S_3' subsites of the enzyme.

Acetonitrile and triethylamine (Aldrich) were dried over freshly activated Linde 3A molecular sieves. ^{1}H and ^{13}C NMR spectra of the synthesized compounds were recorded on a Varian XL-400 NMR spectrometer. A Hewlett-Packard diode array UV-vis spectrophotometer was used in the enzyme assays and inhibition studies. Human leukocyte elastase was purchased fom Elastin Products Co., Owensville, St. Louis. Human leukocyte cathepsin G and proteinase 3 were purchased from Athens Research and Technology Co., Athens, GA. The enzyme substrates methoxysuccinyl Ala-Ala-Pro-Val p-nitroanilide (HLE, PR 3) and methoxysuccinyl Ala-Ala-Pro-Phe p-nitroanilide (Cat G) were purchased from Sigma Chemicals Co., St. Louis, MO.

Representative synthesis

Synthesis of 4-isobutyl-5-benzyl-2-(5-phenyl-2-mercapto-1,3,4-oxadiazolyl)methyl-3-one 1,1 dioxide 10. A solution of 1,8-diazabicyclo[5.4.0]undecen-7-ene (DBU) (0.33 g; 2.2 mmol) and 5-phenyl-2-mercapto-1,3,4-oxadiazole (0.32 g; 2.2 mmol) in dry acetonitrile (10 mL) was stirred for ten minutes at room temperature under nitrogen. 5-Benzyl-2-chloromethyl-4-isobutyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide (0.66 g; 2 mmol) was added and the mixture was refluxed for 2 h. The solvent was removed in vacuo and the residue was taken up in ethyl acetate (70 mL) and washed with water (15 mL), 5% $NaHCO_3$ (15 mL), 5% HCl (15 mL), brine (20 mL) and dried over anhydrous sodium Na_2SO_4. Removal of the solvent left a crude product which was purified using flash chromatography (silica gel) and a hexane/methylene chloride eluent.

Acknowledgements

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