Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2738–2741

The Bsmoc group as a novel scaffold for the design of irreversible inhibitors of cysteine proteases

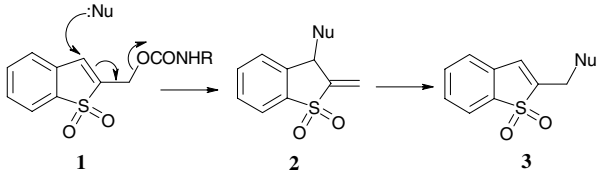
Jim Iley,[a,*] Rui Moreira,[b,*] Luísa Martins,[b] Rita C. Guedes[b] and Cláudio M. Soares[c]

[a]*Chemistry Department, The Open University, Milton Keynes, MK7 6AA, UK*
[b]*CECF, Faculty of Pharmacy, University of Lisbon, Avenida das Forças Armadas, 1600-083, Portugal*
[c]*Instituto de Tecnologia Química e BiológicaUniversidade Nova de Lisboa, 6° Piso, Avenida da Republica, Apartado 127, 2781-901 Oeiras, Portugal*

Received 10 November 2005; revised 6 February 2006; accepted 6 February 2006
Available online 28 February 2006

Abstract—Carbamate and ester derivatives of the 1,1-dioxobenzo[*b*]thiophen-2-ylmethyloxycarbonyl (Bsmoc) scaffold react readily with thiols via a Michael addition at rates not significantly affected by the nature of the carboxylic or carbamic acid leaving group. These Michael acceptors are irreversible inhibitors of the cysteine proteases papain and human liver cathepsin B, displaying first-order kinetics with respect to inhibitor concentration. In contrast, none of the Bsmoc derivatives inhibited porcine pancreatic elastase, a serine protease.

The Bsmoc, 1,1-dioxobenzo[*b*]thiophen-2-ylmethyl-oxycarbonyl, group is a novel, base-sensitive amino protecting group, the usefulness of which is due to deblocking and base scavanging occurring in a single step.[1,2] Deprotection/base scavanging involve Michael addition of a secondary amine, for example, piperidine, to form the intermediate **2**, which undergoes rearrangement to **3**, as confirmed by ^{1}H NMR spectroscopy (Scheme 1).[1] The Bsmoc strategy has been successfully used in the synthesis of a wide range of peptides and amino acid drug derivatives under extremely mild conditions.[2–5] The reactivity of **1** suggests the Bsmoc protecting group to be a particularly attractive Michael acceptor for thiol nucleophiles and thus a potential lead structure for the design of irreversible cysteine protease inhibitors. Michael acceptor scaffolds, such as peptidyl vinylsulfones, sulfonamides or sulfonates,[6–8] have been used to design cysteine protease inhibitors that irreversibly alkylate the active site cysteine residue via conjugate addition.[9] Cysteine proteases represent a broad class of proteolytic enzymes, widely distributed among mammals, protozoa, bacteria, and viruses that are involved in a diverse range of physiological processes, including microorganism–host interaction mechanisms.Unregulated proteolysis by cysteine proteases can lead to many disease states and thus these enzymes are important targets for designing inhibitors as potential therapeutic agents.[10,11]

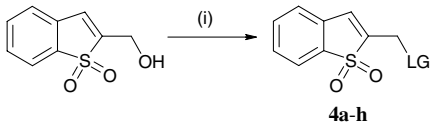


Scheme 1. Mechanism for the deprotection of Bsmoc-amino acids with nucleophiles (Nu, secondary amines).

The recent report[12] of the inclusion of the Bsmoc moiety in dihydroisoxazole inhibitors of human transglutaminase 2, an enzyme believed to play a crucial role in several pathological disorders, and that such inhibitors are unstable in the presence of a thiol such as GSH, prompts us to report our evaluation of the Bsmoc group as Michael acceptor for thiols and its ability to inhibit human liver cathepsin B and papain in an irreversible manner. Cathepsin B is a lysosomal cysteine protease that has been implicated in rheumatoid arthritis and in the progression and invasion of tumors.[10] Cathepsin B degrades extracellular matrix components, either intracellularly or extracellularly, thus enabling the tumor to progress.[10]

Keywords: Papain; Cathepsin B; Cysteine proteases; Bsmoc.
* Corresponding authors. Tel.: +44 1908652513; fax: +44 1908858327 (J.I); tel.: +351 217946477; fax: +351 217946470 (R.M.); e-mail addresses: j.n.iley@open.ac.uk; rmoreira@ff.ul.pt

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.02.007



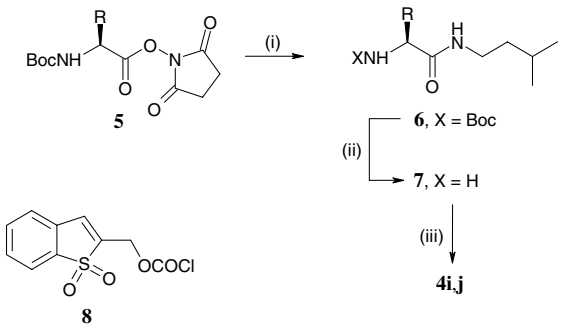
Scheme 2. Reagents: (i) RNCO (for **4a**–**e**) or RCOCl (for **4f**–**h**), TEA, CH_2Cl_2.

Papain is a cysteine protease with a broad range of specificity, sharing sequence and structural homology with other mammalian cysteine proteases.[13]

On the basis of the known chemistry of the Bsmoc group (Scheme 1), we rationalized that compounds **4** (see Scheme 2) with a good leaving group (LG) would inactivate papain via conjugate addition of a cysteine SH group to form **2** (NuH = CysSH) or, by rearrangement, **3**, either of which contain a second Michael acceptor capable of reacting with a second active site nucleophile and thus leading to irreversible inactivation.

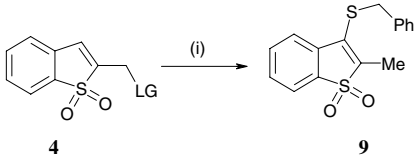
The carbamic acid (**4a**–**e**) or carboxylic acid (**4f**–**h**) Bsmoc derivatives were prepared in good yield by reaction of 1,1-dioxobenzo[*b*]thiophen-2-ylmethanol (**4**, LG = OH) with the appropriate isocyanate or benzoyl chloride, respectively (Scheme 2 and Table 1). The Leu-*i*-Am, **4i**, and Phe-*i*-Am, **4j**, carbamates (Table 1) were also prepared to probe the potential interaction of the amino acid with the S_2 subsite of papain and cathepsin B. Cystein proteases of the papain superfamily prefer substrates with lipophilic amino acids, for example, Phe, in the P_2 position.[9] Moreover, the Leu side chain in the epoxysuccinyl-Leu-*i*-Am inactivator, E64c, has been shown to interact with the S_2 subsite in papain.[9] Compounds **4i**,**j** were prepared from the corresponding Boc-protected aminoacid *N*-hydroxysuccinimide ester, **5**, and *iso*-amylamine, to yield the amides **6** (Scheme 3).[14] After removal of the Boc group, the intermediates **7** reacted with 1,1-dioxobenzo[*b*]thiophen-2-ylmethyl chloroformate, **8**, to give **4i**,**j**.

To assess the intrinsic reactivities of these derivatives, and as a measure of the irreversible chemical step of



Scheme 3. Reagents: (i) $Me_2CHCH_2CH_2NH_2$, TEA, DCM; (ii) TFA, DCM; (iii) **8**, TEA, THF.

enzyme inactivation, we studied their reaction with phenylmethanethiol, dithiothreoitol (DTT), and *N*-acetylcysteine. In contrast to the reaction of Bsmoc-protected amino acids with piperidine, which leads to the rearrangement product **3**, compounds **4** undergo Michael addition with phenylmethanethiol to form **9**[15] along with the corresponding amine or carboxylic acid (Scheme 4). The second-order rate constants for **4a**–**d** correlate (Fig. 1) with the Hammett σ values for the substituents in the arylamino moiety, yielding a ρ value of 0.3 ($r^2 = 0.91$). This indicates that reactivity increases with electron-withdrawing substituents, the relatively low value being consistent with rate-limiting addition of thiol to the cyclic vinylsulfone moiety rather than the carbonyl carbon or exocyclic methylene positions. A similar ρ value can be determined for reaction of **4a**–**d** with DTT (Table 1). Furthermore, the second-order rate constant for the reaction of **4d** with *N*-acetylcysteine (44.4 M^{-1} s^{-1}) is some 20 times greater than the



Scheme 4. Reagents: (i) $PhCH_2SH$, TEA, CH_2Cl_2.

Table 1. Second-order rate constants for the reaction of **4** with DTT (k_2) and for papain inactivation by **4** (k_{obsd}/[I])

Compound	LG	k_2 (M^{-1} s^{-1})	k_{obsd}/[I] (M^{-1} s^{-1})		
			Papain	Cathepsin B	PPE
4a	$OCONHC_6H_4$-4-OMe	4.68	2.15	0.22	NI[a]
4b	$OCONHC_6H_5$	5.68	1.24	0.46	NI
4c	$OCONHC_6H_4$-4-CF_3	8.67	0.64	0.035	NI
4d	$OCONHC_6H_4$-4-NO_2	9.65	ND[b]	ND	NI
4e	$OCONHCH_2C_6H_5$	3.67	0.89	0.45	NI
4f	$OCOC_6H_5$	7.82	2.48	0.030	NI
4g	$OCOC_6H_4$-4-OMe	7.68	0.45	0.10	NI
4h	$OCOC_6H_4$-2-OMe	7.80	1.60	0.24	NI
4i	OCO-Leu-NH-iAm	6.65	0.93	0.14	ND
4j	OCO-Phe-NH-iAm	7.10	0.65	0.085	ND
Ac-Phe-NH-CH_2-CH=CH-SO_2Me[c]			18.7		

[a] No inhibition.
[b] Not determined.
[c] Ref. 16.

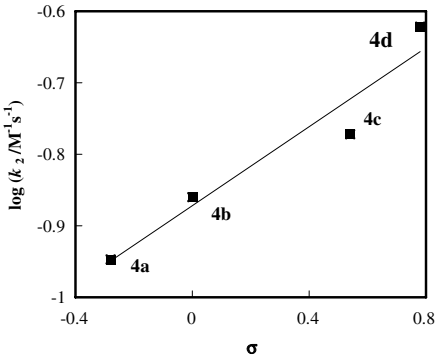


Figure 1. Hammett plot for the reaction of Bsmoc derivatives **4a**–**d** with $PhCH_2SH$ at 25 °C.

corresponding value for the reaction with piperidine (2.1 M^{-1} s^{-1}), the reagent recommended for removing the Bsmoc protecting group, indicating a significantly greater reactivity of the Bsmoc scaffold with the thiol functionality than with an amine. Additionally, there is no major difference between the reactivity of carbamates **4a**–**e** and esters **4f**–**h**, as would be expected if reaction were occurring at the ring C-3, rather than the carbonyl, position. Indeed, the sterically hindered 2-MeO-substituted ester **4h** reacts with DTT at an almost identical rate to that of its 4-MeO counterpart **4g** (Table 1), which is also consistent with conjugate addition of the thiol to the vinylsulfone.

The inhibitory potency of compounds **4** against papain and cathepsin B was assessed using Kitz and Wilson's incubation method.[17] Both Bsmoc carbamate, **4a**–**e** and **4i**,**j**, as well as ester, **4f**–**h**, derivatives, were found to be time-dependent inactivators of papain (Fig. 2) and cathepsin B. Pseudo-first-order rate constants of inactivation, k_{obsd} values, were obtained from plots of % activity (v/v_0) against time.[18] For each assay, the concentration of each Bsmoc derivative was normalized to take account of the competitive reaction with DTT.[18,19] The irreversible nature of the inactivation was shown in a routine assay, when no reactivation of enzyme activity was detected even after **4d** of dialysis at 25 °C.

For the enzyme inhibition experiments, due to solubility problems at the higher values of inhibitor concentration

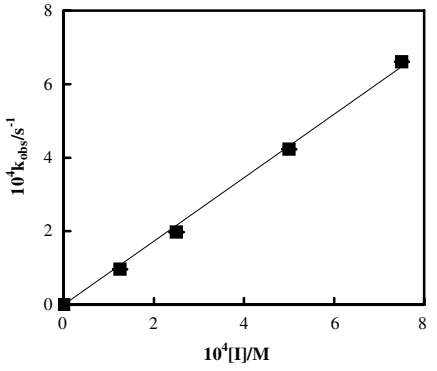


Figure 2. Plot of the first-order rate constant, k_{obsd}, for the inactivation of papain **4b** against inhibitor concentration.

[I], neither K_I nor k_{inact} (the first-order rate constant for the chemical inactivation step) could be determined for papain or cathepsin B. However, the corresponding second-order rate constants for inactivation, k_2 (=k_{inact}/K_I), were determined from the slopes of the plots of k_{obsd} versus [I] and are expressed as k_{obsd}/[I], ranging from 0.35 to 2.48 M^{-1} s^{-1} for papain (Table 1). These values indicate that both carboxylate and carbamic acid leaving groups lead to active compounds and contrast with the value of 0.08 M^{-1} s^{-1} determined for the alcohol starting material (**4**, LG = OH). Such 6- to 35-fold difference between the second-order inactivation constants for compounds **4a**–**h**, when compared to the alcohol counterpart, can be ascribed, in part, to the increased electron-withdrawing effect of the carbonyloxymethyl moiety when compared to the hydroxyl substituent (the Taft σ^* values for $OCOC_6H_5$ and OH are 2.57 and 1.34, respectively). However, the most potent papain inhibitor in the carbamate series is the 4-MeO substituted compound **4a**, which is ca. 3 more active than the 4-CF_3 analogue **4c**. Unfortunately, introducing the Leu-*i*-Am and Phe-*i*-Am side chains, (**4i** and **4j**, respectively) did not improve the inhibitory potency when compared to carbamates and esters **4a**–**h**. Despite being efficient Michael acceptors, Bsmoc derivatives **4a**–**j** are ca. 8 to 40 times less potent than vinylsulfone Ac-$PheNHCH_2CH{=}CHSO_2Me$ (Table 1).[16]

Compounds **4** are also time-dependent inhibitors of cathepsin B,[20] but the corresponding k_{obsd}/[I] values are 2–80 times smaller than those for papain. The most potent compounds in the series, **4a** and **4f**, are also the most selective for papain. Interestingly, the most active compound against papain, **4f**, is the least active against cathepsin B. As for papain, the Leu-*i*-Am and Phe-*i*-Am side chains did not improve inhibition against cathepsin B. The Bsmoc derivatives **4** were also tested against porcine pancreatic elastase (PPE),[21] a serine protease, using the dilution assay. None of the Bsmoc derivatives inhibited PPE up to a concentration of 10 mM, which suggests that **4** are selective inhibitors for cysteine proteases (Table 1).

We have yet to examine the precise nature of the interaction of the enzyme and the Bsmoc derivatives using mass spectrometry. However, modeling the interaction of **4f** with papain (pdb: 1PPN) using Autodock software[22] reveals the preferred conformation of the inhibitor to sit in the active site such that the pro-*S* sulfonyl oxygen atom forms a hydrogen bond to the NH of Gly-66, while the benzoate moiety sits in the S_2 pocket of the enzyme. This orientation presents C-3 of the benzothiophene-1,1-dioxide system to the sulfur atom of Cys-25 of the Asn-175/His-159/Cys-25 catalytic triad, as would be expected for Michael addition chemistry as described above (Fig. 3).

The results herein presented show that the Bsmoc scaffold provides a novel and unexplored approach for achieving cysteine protease inhibiton. The Bsmoc-derived carbamates and esters **4** react with thiols *via* conjugated addition to the cyclic vinylsulfone moiety. This pathway is likely to be identical to that of

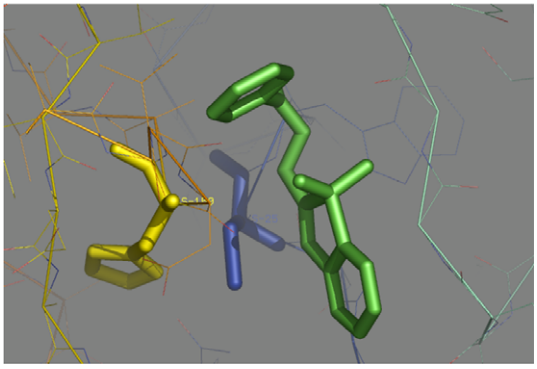


Figure 3. Autodock-generated active site interaction of papain and **4f** (green). His-159 (blue) and Cys-25 (yellow) are also highlighted. Figure generated using Pymol software: DeLano, W.L. The PyMOL Molecular Graphics System (2002) DeLano Scientific, San Carlos, CA, USA. http://www.pymol.org.

alkylation of the active site cysteine that leads to papain and cathepsin B inactivation.

Acknowledgments

We thank FCT, POCTI, and FEDER (Portugal) for financial support and a grant to L.M. (SFRH/BD/6499/2001).

References and notes

1. Carpino, L. A.; Philbin, M.; Ismail, M.; Truran, G. A.; Mansour, E. M. E.; Iguchi, S.; Ionescu, D.; El Faham, A.; Riemer, C.; Warrass, R.; Weiss, M. S. *J. Am. Chem. Soc.* **1997**, *119*, 9915.
2. Carpino, L. A.; Ismail, M.; Truran, G. A.; Mansour, E. M. E.; Iguchi, S.; Ionescu, D.; El Faham, A.; Riemer, C.; Warrass, R. *J. Org. Chem.* **1999**, *64*, 4324.
3. Greenwald, R. B.; Zhao, H.; Reddy, P. *J. Org. Chem.* **2003**, *68*, 4894.
4. Carpino, L. A.; Krause, E.; Sferdean, C. D.; Schümann, M.; Fabian, H.; Bienert, M.; Beyermann, M. *Tetrahedron Lett.* **2004**, *45*, 7519.
5. Carpino, L. A.; Ghassemi, S.; Ionescu, D.; Ismail, M.; Sadat-Aalaee, D.; Truran, G. A.; Mansour, E. M. E.; Siwruk, G. A.; Eynon, J. S.; Morgan, B. *Org. Process Res. Dev.* **2003**, *7*, 28.
6. Palmer, J. T.; Rasnik, D.; Klaus, J. L.; Brömme, D. *J. Med. Chem.* **1995**, *38*, 3193.
7. Roush, W. R.; Gwaltney, S. L.; Cheng, J.; Scheidt, K. A.; McKerrow, J. H.; Hansell, E. *J. Am. Chem. Soc.* **1998**, *120*, 10994.
8. Reddick, J. J.; Cheng, J.; Roush, W. R. *Org. Lett.* **2003**, *5*, 1967.
9. Powers, J. C.; Asgian, J. L.; Ekicu, Ö. D.; James, K. E. *Chem. Rev.* **2002**, *102*, 4639.
10. Otto, H.-H.; Schirmeister, T. *Chem. Rev.* **1997**, *97*, 133.
11. Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305.
12. Choi, K.; Siegel, M.; Piper, J. L.; Yuan, L.; Cho, E.; Strnad, P.; Omary, B.; Rich, K. M.; Khosla, C. *Chem. Biol.* **2005**, *12*, 469.
13. Meara, J. P.; Rich, D. H. *J. Med. Chem.* **1996**, *39*, 3357.
14. Roush, W. R.; Hernandez, A. A.; McKerrow, J. H.; Selzer, P. M.; Hansell, E.; Engel, J. C. *Tetrahedron* **2000**, *56*, 9747.
15. Salient data for **9** are as follows: ^{1}H NMR (300 MHz, CD_3CN) 1.91 (3H, s, CH_3Ar), 3.96 (2H, s, CH_2Ph), 7.08–7.75 (9H, m, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$) 8.41 (CH_3Ar), 38.73 (CH_2Ph), 121.05, 122.80, 127.58, 128.57, 128.64, 129.36, 132.32, 132.60, 133.52, 135.64, 136.50, 144.95 (Ar). $C_{16}H_{12}O_2S_2$ requires: C, 63.55; H, 4.67%. Found: C, 63.50; H, 4.70%.
16. Liu, S.; Hanzlik, R. P. *J. Med. Chem.* **1992**, *35*, 1067.
17. Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245.
18. Papain (EC 3.4.22.2, from Sigma) assays were performed as described in Zhao, G.; Zhou, Z. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2331, Gradients from inhibitor runs were normalized with respect to the first-order exponential decrease in Bsmoc inhibitor concentration due to DTT-catalyzed decomposition at the pH of the incubation.[19] Observed pseudo-first-order rate constants of inactivation, k_{obsd}, were obtained from plots of % activity against time.
19. Hinchliffe, P. S.; Wood, J. M.; Davis, A. M.; Austin, R. P.; Beckett, R.; Page, M. I. *Org. Biomol. Chem.* **2003**, *1*, 67.
20. Human cathepsin B (EC 3.4.22.1, from Calbiochem) assays were carried out as described for papain using pH 5.5, 50 mM acetate buffer (2 mM DTT, 2 mM EDTA). The substrate was Z-Arg-Arg-NA (0.1 mM).
21. PPE (EC 3.4.21.36, from Sigma) inhibition assays were carried out as described in Ref. 19.
22. AutoDock 3.0; automated docking using a lamarckian genetic algorithm and empirical binding free energy function Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comp. Chem.* **1998**, *19*, 1639.