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Dipeptide vinyl sultams: Synthesis via the Wittig–Horner reaction and activity against papain, falcipain-2 and *Plasmodium falciparum*

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Abstract—The synthesis of phosphonate derivatives of *N*-phenyl- and *N*-benzyl-γ- and δ-sultams, and their application in the Wittig-Horner reaction with *N*-Boc-L-phenylalanine aldehyde to afford *E*- and *Z*-isomers, are described. These compounds were further processed to provide five dipeptide vinyl sultams, which were found to be inactive against papain at concentrations up to 50 μM. In contrast, vinyl sultams demonstrated weak activity against recombinant falcipain-2 and *Plasmodium falciparum* W2.

Malaria is the major life-threatening parasitic disease in tropical and sub-tropical regions. Worldwide, there are at least 300 million acute cases of malaria and more than 1 million deaths each year, mostly young children infected with *Plasmodium falciparum*.[1] With the rapid spread of multidrug-resistant *P. falciparum* strains, the development of safe and effective antimalarials has become an important strategy towards achieving effective control of malaria. Cysteine proteases regulate a broad spectrum of physiological functions in mammals, parasitic protozoa, plants and yeast. In humans, elevated levels of these enzymes can lead to disease states such as osteoporosis, rheumatoid arthritis and cancer.[2,3] In parasites, they play a crucial role in metabolism and reproductive function.[2,3] Falcipain-2 is a cysteine protease stored in the acidic food vacuole of *P. falciparum* and is likely involved in the hydrolysis of haemoglobin that produces free amino acids required for parasite survival.[4–6] Disruption of this haemoglobin degradation pathway is lethal to the parasite and thus compounds designed to inhibit falcipain-2 present excellent opportunities for developing antimalarial drug candidates.

Vinyl sulfones (VS) and their analogues, such as sulfonamides and sulfonates, have been reported as a promising class of inhibitors for parasitic cysteine proteases. They act by irreversibly alkylating the active site cysteine residue via conjugate addition.[2,3,7]

Developing conformationally restricted analogues is a commonly used approach to probe the binding site of receptors and to further improve activity.[8] Despite the recent finding that cyclic vinyl sulfones derived from the Bsmoc amino-protecting group are irreversible inhibitors of papain and cathepsin B,[9] the design of conformationally restricted analogues of sulfones as cysteine protease inhibitors is still an underexplored field. In this context, we now report the synthesis of the vinyl sultam scaffold as a cyclic isostere of vinyl sulfonamides, and thus as a building block for a new family of irreversible cysteine protease inhibitors. Vinyl sultams containing an exocyclic double bond are also structurally similar to α-methylene lactones, which are known Michael acceptors for thiols.[10,11] With the aim of designing effective inhibitors for falcipain-2, the vinyl sultams were attached to a peptide sequence. The chosen sequence was Mu-Leu-Phe (Mu = 4-morpholinecarbonyl), by analogy with the vinyl sulfone Mu-Leu-Hph-VS-Ph, which was considered an optimal inhibitor for this enzyme (IC_{50} 0.003 μM).[2] A vinyl sultam with the Ac-Phe-Phe moiety was also prepared based on the success of the vinyl sulfones Cbz-Phe-Hph-VS-R against cruzain, a related cysteine protease from *Trypanosoma*

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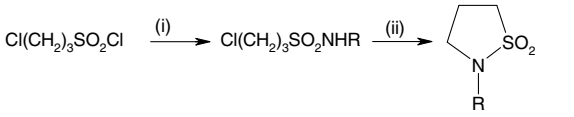
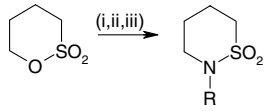
cruzi.[2] The sultam nitrogen atom carried either a benzyl or phenyl group. The evaluation of the target compounds against falcipain-2, papain and the chloroquine-resistant *P. falciparum* strain W2 is also reported.

Synthesis involved the preparation of the γ- and δ-sultam cores **1** and **2** (Schemes 1 and 2), using appropriate modifications of previously described methods.[12,13] Incorporation of the peptide moiety proceeded according to the general sequence reported for the analogous vinyl sulfones,[14] which involve as the key step a Wittig-Horner reaction (Scheme 3). This method has been widely applied to the synthesis of α,β-unsaturated sulfonate derivatives,[6,14,15] but to the best of our knowledge, not to sultams; reported synthesis of vinyl derivatives of the latter involves: (i) the direct coupling by an aldol-like reaction, followed by dehydration[16] and (ii) the intramolecular Heck reaction.[17] In order to follow the Wittig-Horner approach, the anion of **2a** was generated (2 equiv *n*-BuLi, dry THF, rt, N_2) and allowed to react with diethyl chlorophosphate to afford compound **4a** (method A).[15,18] However, when this procedure was applied to the other sultams it furnished either decomposition or poor yields (Table 1). For these reasons, conditions B and C were investigated. In general, we found LDA at −78 °C to afford higher yields of the sultam phosphonates than BuLi either at −78 °C or room temperature. Phosphonates **3** and **4** were condensed with *N*-Boc-L-phenylalanine aldehyde (**5**), prepared from *N*-Boc-L-phenylalanine via formation of the *N*,*O*-dimethylhydroxamate.[19] The reaction provided *E*- and *Z*-vinyl sultams **6**–**9** (Table 2). The *E*/*Z* ratio is consistent with the general observation that phosphonates with alkyl groups give mostly the thermodynamically more stable *E*-olefins.[20,21]



1a R = Ph, 49% (2 steps)
1b R = Bz, 79% (2 steps)

Scheme 1. Reagents and conditions: (i) $PhNH_2$, TEA, DCM, reflux, 1 h or $BzNH_2$, DCM, −10 °C, 1 h; (ii) NaOH, TEA, MeOH, reflux.



2a R = Ph, 43% (3 steps)
2b R = Bz, 44% (3 steps)

Scheme 2. Reagents and conditions: (i) RNH_2, 100 °C; (ii) $POCl_3$, reflux; (iii) K_2CO_3, AcOEt, rt.

The geometry of the double bond comes from the examination of the chemical shifts of vinyl and methine protons (Table 3). This process has been reported as a reliable criterion for stereochemical assignment of α,β-unsaturated sulfonates:[15] as a result of the deshielding effect of the sulfonyl group, the vinyl proton resonance

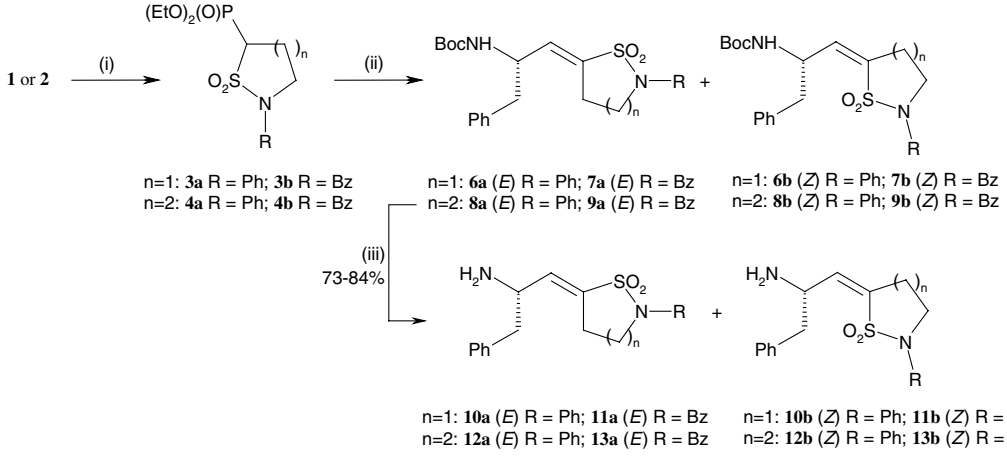
Table 1. Conditions for the synthesis of sultam phosphonates **3** and **4**

Phosphonate	Yield (%)		
	Method A	Method B	Method C
3a	27	56	65
3b	0	—	43
4a	48	—	45
4b	15	—	34

Method A: *n*-BuLi, dry THF, rt, N_2; method B: *n*-BuLi, dry THF, −78 °C, N_2; method C: LDA, dry THF, −78 °C, N_2.

Table 2. Yields and ratio of *E*/*Z* isomers

Phosphonate	Product	*E*/*Z*	Yield (%)	*E*/*Z* ratio
3a	**6a**	*E*	60	79:21
	6b	*Z*	16	
3b	**7a**	*E*	58	67:33
	7b	*Z*	29	
4a	**8a**	*E*	65	90:10
	8b	*Z*	7	
4b	**9a**	*E*	54	86:14
	9b	Z	9	



n=1: **3a** R = Ph; **3b** R = Bz
n=2: **4a** R = Ph; **4b** R = Bz

n=1: **6a** (*E*) R = Ph; **7a** (*E*) R = Bz
n=2: **8a** (*E*) R = Ph; **9a** (*E*) R = Bz

n=1: **6b** (*Z*) R = Ph; **7b** (*Z*) R = Bz
n=2: **8b** (*Z*) R = Ph; **9b** (*Z*) R = Bz

n=1: **10a** (*E*) R = Ph; **11a** (*E*) R = Bz
n=2: **12a** (*E*) R = Ph; **13a** (*E*) R = Bz

n=1: **10b** (*Z*) R = Ph; **11b** (*Z*) R = Bz
n=2: **12b** (*Z*) R = Ph; **13b** (*Z*) R = Bz

Scheme 3. Reagents and conditions: (i) LDA, THF, −78 °C, $(EtO)_2POCl$, N_2; (ii) *n*-BuLi, THF, Boc-Phe-CHO (**5**), −78 °C to rt, N_2; (iii) TFA 50% (w/v DCM solution), rt, 20 min.

Table 3. Chemical shifts δ (parts per million) and coupling constants J (hertz) in $CDCl_3$ for vinyl and methine protons of E/Z isomers

Compound	E/Z	δ C*H*=	δ C*H*CH_2Ph
6a	*E*	6.31 br d (8.8)	4.53 br s
6b	*Z*	6.35 br s	4.91 m
7a	*E*	6.29 br d (9.2)	4.48 m
7b	*Z*	6.26 br s	4.96 m
8a	*E*	6.24 br d (8.8)	4.71 m
8b	*Z*	6.12 br s	5.10 br s
9a	*E*	6.21 d (9.6)	4.68–4.74 m
9b	*Z*	5.98 br s	5.17 br s

is found downfield in the *E*-isomer compared to the *Z*-isomer, whereas methine proton signal appears upfield. Stereochemistry is further supported by means of NOESY spectra, on the basis of a NOE cross-peak between the vinyl and =CCH_2 protons in the *Z*-isomer **9b**.

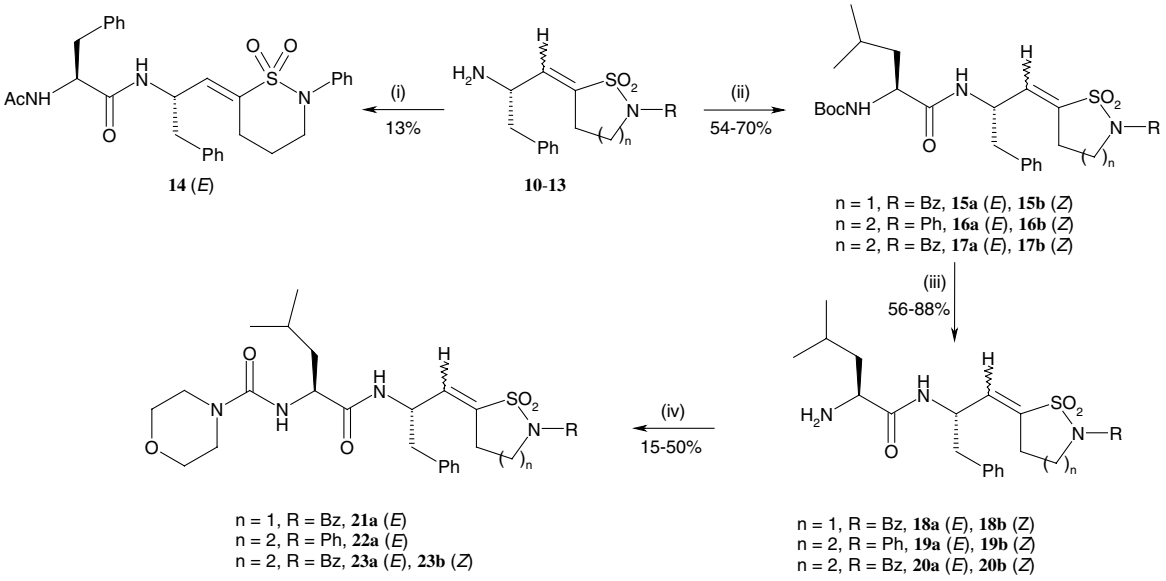
To synthesize the dipeptide vinyl sulfones (**14** and **21**–**23**), the *N*-Boc group of **6**–**9** was easily cleaved with trifluoroacetic acid and the resulting amines were coupled with Boc-protected amino acids under standard peptide coupling conditions (Scheme 4).[22] These compounds were screened for effectiveness against papain, the archetype of cysteine proteases. No inhibition could be detected at concentrations up to 50 μM. However, when evaluated against falcipain-2, they showed some activity, albeit modest (Table 4). Compound **23a** exhibited the best IC_{50} value (13.7 μM), which was consistently accompanied by the most favourable activity against *P. falciparum* W2. Though the range of compounds is limited, the data presented suggest that the ring size (**21a** vs **23a**), the geometry of the double bond (**23a** vs **23b**) and the nitrogen substituent (**22a** vs **23a**) may have some impact on activity. As shown by the data for compounds **14** and **22a** substitution of the AcPhe by the Mu-Leu moiety has little effect on activity.

Table 4. Biological activity of dipeptide vinyl sultams

Compound	Papain[a]		IC_{50}[c] (μM)	
	[I] μM	% of inhibition	rec-FP-2	*P. falciparum* W2
9a	50	NI	—	—
14	50	NI[b]	26.5	>10
21a	—	—	48.4	>10
22a	—	—	31.4	>10
23a	50	NI	13.7	7.8
23b	50	NI	23.6	>10
24[d]	—	—	0.003[e]	0.2[f]

[a] Assays with papain using the incubation method.[24]
[b] No inhibition after 30 min of incubation.
[c] Assays of falcipain inhibition and parasite development were determined as described earlier.[6]
[d] Compound **24** = Mu-Leu-hPh-VSPh.
[e] Non-recombinant falcipain.[25]
[f] Itg2 strain.[25]

Overall, what is immediately clear from these results is that dipeptide vinyl sultams are significantly less active (≈10^{-4}-fold) than their acyclic analogues, vinyl sulfones. This result was totally against expectation, especially given a recent report which shows that bicyclic α-methylene-γ-sultams are reactive towards sulfur nucleophiles.[17] A possible explanation is that the α-carbon of Michael acceptors **14** and **21**–**23** incorporates the ring moiety, interfering with enzyme binding and leading to a substantial reduction in the activity when compared to their acyclic counterparts, in which the α-carbon is not substituted.[23] To get some insight into the binding mode of vinyl sultams to cysteine proteases, we studied the molecular interactions between the more potent inhibitors **23a**,**b** and cruzain, using the program GOLD.[26] Falcipain-2 and cruzain share more than 40% homology in the mature domain and about 90% for the binding site residues.[27,28] We chose a cruzain structure co-crystallized with vinyl sulfone CbzPhe-hPhe-SO_2CH_2Ph, **25**, via a covalent bond (PDB code: 1F2A). Each ligand was energy-minimized and then



Scheme 4. Reagents and conditions: (i) **12a**, *N*-acetyl-L-phenylalanine, TEA, HOBt, DCC, DCM, rt, three days; (ii) *N*-Boc-L-leucine-*N*-hydroxysuccimide ester, TEA, THF, rt, 14–48 h; (iii) TFA 50% (w/v DCM solution), rt, 20 min; (iv) 4-morpholinecarbonyl chloride, TEA, THF, rt, 14–16 h.

subjected to 50 docking runs. The top 10 solutions (i.e., those with the highest fitness score) were visually analyzed for: (i) the hydrophobic and hydrophilic interactions between the ligand and enzyme surfaces and (ii) the distance between the β-carbon atom of the Michael acceptor system and the catalytic cysteine residue. Modelling the interaction of vinyl sulfone **25** with cruzain revealed that seven conformations presented hydrogen bonding interactions between the sulfonyl oxygen atoms and the imidazole NH of His159, indole NH of Trp177 and one of the NH of Gln19, while all 10 inspected conformations present the Phe side chain sitting in the S2 pocket of the enzyme. Additional hydrogen bonding to the carbonyl oxygen atom of Gly66 enables further stabilization of the non-covalent complex. This conformation presents the β-carbon atom of the vinyl sulfone system in proximity (3.6–3.8 Å) to the sulfur atom of Cys25, as would be expected for Michael addition chemistry.

Interestingly, docking *E*-isomer **23a** to cruzain revealed that the top ranking conformation sits in the active site with an inverted orientation when compared to **25**, the *N*-benzyl-δ-sultam moiety being accommodated in the S2 subsite through stabilizing van der Waals interactions with Leu67, Ala133 and Leu157 (Fig. 1A). The most striking observation was that the sulfur atom of Cys25 is in the vicinity (3.4 Å) of the β-carbon and positioned 20° out off of the plane defined by the carbon–carbon double bond, thereby disfavouring Michael addition of cysteine to the β-carbon atom of the vinyl sultam system. This type of reaction requires the sulfur nucleophile to approach the carbon atom almost perpendicularly to the double bond plane in order to achieve optimal orbital overlap. In contrast, the preferred conformation of the *Z*-isomer **23b** is positioned in the active site of cruzain similarly to **25**, that is, with the Leu side chain occupying the S2 pocket (Fig. 1B). However, the Cys25(*S*)-Cβ distance is probably too long (ca. 4.0 Å) for nucleophilic attack of cysteine to take place. Taken together, these docking results suggest that the δ-sultam ring increases the degrees of freedom of ligand binding to cruzain when compared with the acyclic vinyl sulfone analogue, leading to enzyme–inhibitor complexes that do not favour Michael addition chemistry.

Docking **23a** to papain (PDB code: 1PPN) revealed that seven of the top 10 solutions present the *N*-benzyl-δ-sultam moiety being incorporated in the S2 subsite defined by Pro68, Val133 and Val157, and a Cys25(*S*)-Cβ distance ranging from 4.6 to 4.9 Å. Finally, all docked conformations of **23b** show the *N*-benzyl-δ-sultam in the S2 pocket. Clearly, these results suggest that molecular recognition of **23a**,**b** by papain leads to enzyme–inhibitor complexes that are conformationally unfavourable to conjugate addition of Cys25 to the vinyl sultam, as required for enzyme inactivation.

In summary, this paper describes the synthesis of vinyl sultam derivatives via the Wittig–Horner reaction. Although weakly active, vinyl sultams are selective for falcipain-2 over papain. Docking studies suggest that two different binding modes of vinyl sultams to cysteine proteases are available, neither of which is favourable for the conjugate addition of Cys25 to the vinyl sultam system.

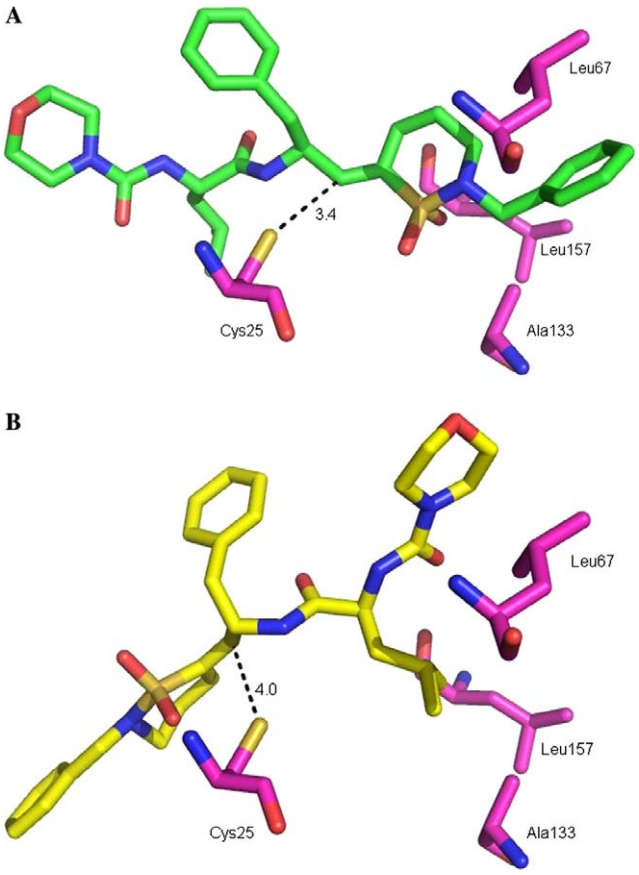


Figure 1. GOLD-generated active site interaction of cruzain and **23a** (A) and **23b** (B); Cys25 and the S2 pocket residues are in magenta. Figure generated using Pymol software: DeLano, W.L. The PyMOL Molecular Graphics System (2002) DeLano Scientific, San Carlos, CA, USA. http://www.pymol.org.

Acknowledgments

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Supplementary data

Supplementary data associated with this article (synthesis procedures, spectroscopic data and molecular modelling details) can be found, in the online version, at doi:10.1016/j.bmcl.2006.04.079.

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