Bioorganic & Medicinal Chemistry Letters 18 (2008) 4210–4214

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of novel 2-pyridone peptidomimetic falcipain 2/3 inhibitors

Edite Verissimo [a,b], Neil Berry [b], Peter Gibbons [b], M. Lurdes S. Cristiano [a], Philip J. Rosenthal [c], Jiri Gut [c], Stephen A. Ward [d], Paul M. O'Neill [b,*]

[a] Department of Chemistry, Biochemistry and Pharmacy, and CCMAR, University of the Algarve, Campus de Gambelas, Faro 8005-039, Portugal
[b] Department of Chemistry, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK
[c] Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA 94143-0811, USA
[d] Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK

ARTICLE INFO

Article history:
Received 8 March 2008
Revised 15 May 2008
Accepted 16 May 2008
Available online 20 May 2008

Keywords:
Malaria
Plasmodium falciparum
Cysteine protease
Falcipain-2
Peptidomimetic

ABSTRACT

The structure-based design, chemical synthesis and in vitro activity evaluation of various falcipain inhibitors derived from 2-pyridone are reported. These compounds contain a peptidomimetic binding determinant and a Michael acceptor terminal moiety capable of deactivating the cysteine protease active site.

Due to increasing resistance to existing antimalarials[1] development of new safe and effective antimalarial drugs remains an important goal toward achieving control of malaria. Among potential new targets for antimalarial chemotherapy are cysteine proteases, enzymes that cleave small peptide substrates by the action of a nucleophilic cysteine thiol group and that in malaria parasites play important physiological roles.[2] The differences in nature of parasitic cysteine proteases and its human orthologues have prompted interest in this class of proteases.[2] Also, it has been shown that cysteine protease inhibitors arrested the erythrocytic life cycle of *Plasmodium falciparum*, the most virulent human malaria parasite, by blocking the hydrolysis of the host hemoglobin, causing abnormally swollen food vacuoles[3] and moreover, some of these molecules have led to cure of malaria in mice.[4,5] *P. falciparum* parasites contain four typical papain family cysteine proteases known as falcipains.[6] The importance of the role of falcipain-1 (FP1) is yet not clear,[6] and the biological role of falcipain-2′ (FP2′), biochemically very similar to falcipain-2 (FP2), is still uncertain too.[7] Better known proteases are falcipain-2[8,9] and falcipain-3 (FP3),[9,10] expressed in trophozoites, localized in the food vacuole and able to degrade hemoglobin, therefore suggesting to be major hemoglobinases and targets for cysteine protease inhibitors.

Initial studies with generic peptide cysteine protease inhibitors like leupeptin and E64 were followed by the finding of more specific ones and several peptide-based falcipain inhibitors have since been identified, including carbonyl-containing[11] and vinyl sulfone-containing inhibitors **1a,b** (Fig. 1).[12] These compounds act by irreversible alkylation of the cysteine residue[13–15] at the active site via either 1,2-addition[16] in the case of aldehydes[17] or conjugate addition in the case of vinyl sulfones. Consistent structural features leading to achieve maximal FP2 and FP3 inhibition by the peptidyl analogues appear to include a leucine residue at the P2 position[8,10,18] and the unnatural amino-acid homophenylalanine at the P1 position.[16,18] Also, conformationally constrained vinyl sulfones **2** (Fig. 1) have been prepared and demonstrate high FP2 inhibition.[19] However, peptidyl inhibitors may have a limited utility as therapeutic agents since they have some theoretical disadvantages, including relatively poor pharmacokinetic profiles as their amide bonds are susceptible to cleavage by other proteases,[20] and also because they can be relatively non-selective and thus promote host toxicity.[21]

The incorporation of a non-peptidic scaffold that can constrain the amino-acid backbone has potential advantages, such as increasing selectivity by stabilizing a biologically active conformation and decreasing toxicity to the human host. Examples of success in the field of peptidomimetics active against cysteine proteases include the discovery of novel peptide-based ICE

* Corresponding author.
E-mail address: P.M.oneill01@liv.ac.uk (P.M. O'Neill).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.05.068

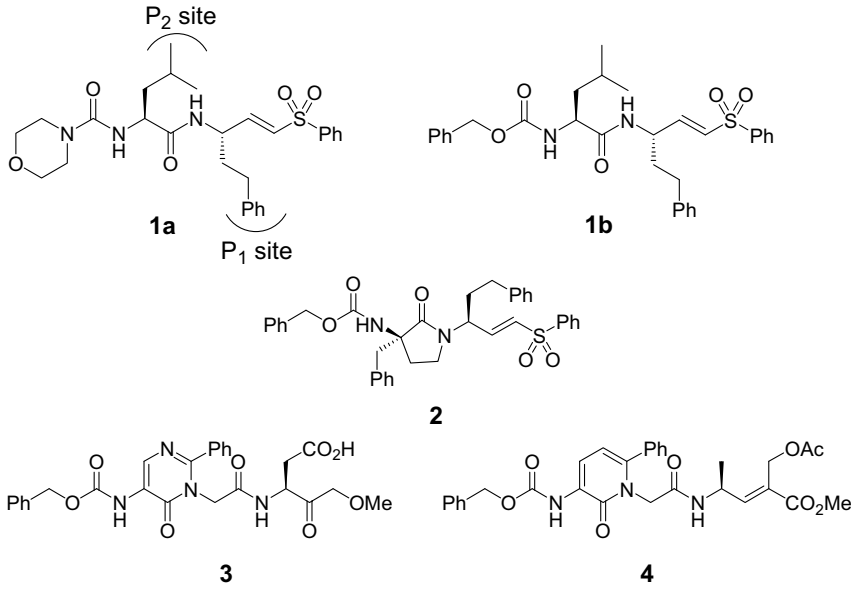


Figure 1. Potent small molecule cysteine protease inhibitors of peptidic and peptidomimetic nature.

(interleukin-1β-converting enzyme) inhibitors **3** (Fig. 1)[22] and novel human rhinoviruses inhibitors.[23] More significantly, studies by Lin et al. at the Walter Reed Army Institute of research showed that peptidomimetic analogues **4** (Fig. 1) can be designed with potent antimalarial activity in vitro.[21] In all these studies, the pyridone ring core was found to be a suitable surrogate for a portion of the peptide.

As the bioactive conformation of the inhibitors (e.g. **1b**) are not known, we undertook a modeling study to verify that the proposed inhibitors **6a** and **6c** could adopt similar low energy conformations to the known inhibitor **1b**. Similarity between **1b** and **6c** was assessed using FieldTemplater, a tool for comparing molecules using their electrostatic and hydrophobic fields.[24] Each conformation of one molecule was aligned to each conformation of the other molecule to identify the most similar compound on the basis of field similarity and volume of steric overlap. As can be seen from Figure 2, the most similar conformations of the proposed inhibitor **6c** and the known inhibitor **1b** possessed a high degree of similarity (Field Similarity 0.789, Volume Similarity 0.863). There were many other 'good' matches of conformation between these two molecules indicating that these molecules could adopt similar low energy conformations. The proposed molecule **6a** was assessed for its similarity to the α,β unsaturated methyl ester analogue of **1b**. Pleasingly, there was a high degree of similarity between **6a** and the α,β unsaturated methyl ester analogue (Field Similarity 0.800, Volume Similarity 0.846), with many other 'good' matches of low energy conformers.

The synthesis of cysteine protease inhibitors described in this study involves incorporation of a 2-pyridone ring and different P1 fragments for recognition and binding to the enzyme and of different Michael acceptor groups capable of reacting with the nucleophilic thiol of the cysteine residue. In this communication, we focus on conformationally restricted peptidomimetic inhibitors **5a,b** and **6a–c** of *P. falciparum* cysteine proteases and the assessment of their antimalarial activity.

Our original synthesis of peptidomimetics was developed in order to achieve target molecules unsubstituted at the P1 position (Lin's approach), by direct use of methylbromoacetate as one of the building blocks (R^1 = H). Later on we introduced a substituent at this position by working from commercially available protected D-phenylalanine and D-homophenylalanine ($R^1 = CH_2Ph$, $R^1 = C_2H_4Ph$).

Preparation of the key intermediate **12** (Fig. 3) was achieved by coupling an amino-protected pyridone ring with a moiety containing the desired substituent at the P1 position and an ester function, to allow subsequent chemical transformations. Pyridonyl-aldehydes **5**, themselves target molecules in this synthesis, were obtained directly from **12**, and the remaining target molecules **6a–c**

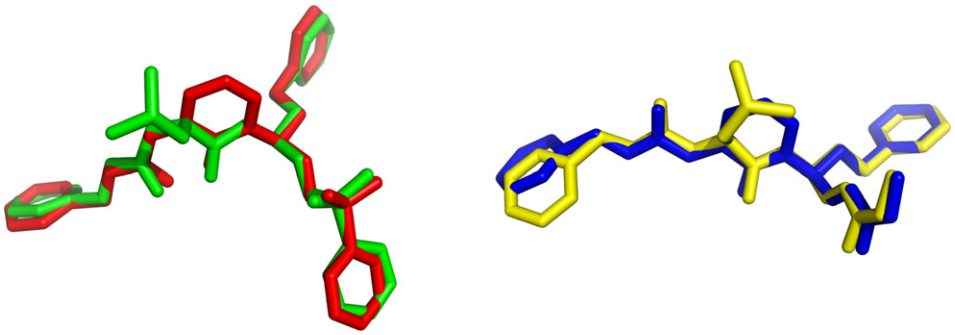


Figure 2. Pairwise molecular alignment from FieldTemplater of **1b** (green) with **6c** (red) and the **1b** α,β unsaturated methyl ester analogue (yellow) with **6a** (blue). (Hydrogens omitted for clarity. Pictures generated using PyMol[25].) The template structure presented in Figure 3 (**6a–c**), designed on the basis of the considerations described above and of molecular modeling studies, represents our target peptidomimetic malarial cysteine protease inhibitor.

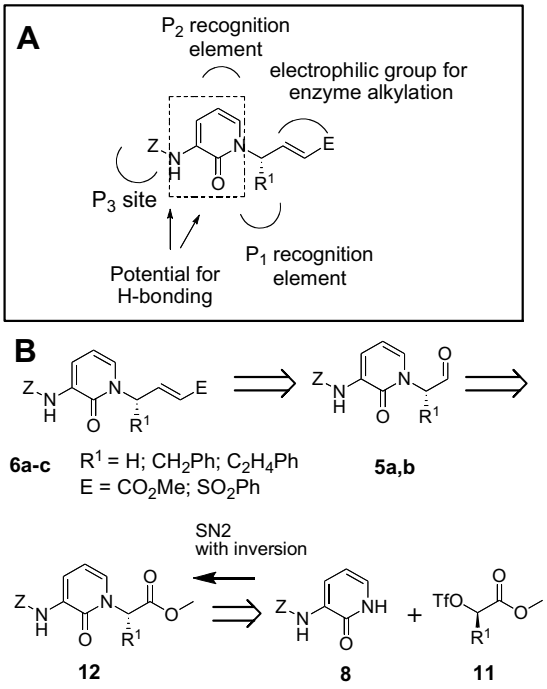


Figure 3. (A) Structure of peptidomimetic template and (B) retrosynthetic analysis for analogues **6a–6c**.

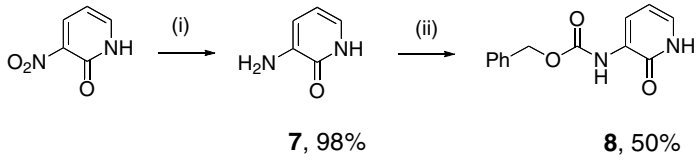
were obtained via a Wittig-type strategy. The synthetic transformations employed followed previously reported preparations of related peptidyl and peptidomimetic inhibitors.[23,26] The pyridone **8** was obtained from commercially available 3-nitropyrid-2-one, by selective reduction of the nitro group and subsequent protection of the primary amine **7** as a carbamate (Scheme 1).

The P1 block **11** was obtained from commercially available Boc-D-phenylalanine and Boc-D-homophenylalanine (Scheme 2). Boc-deprotection followed by nitrous deamination afforded α-hydroxyacid intermediates **9a** and **9b**. Esterification under acidic conditions gave the corresponding methyl esters **10a** and **10b**, which were then converted into the more reactive triflates **11a** and **11b**.

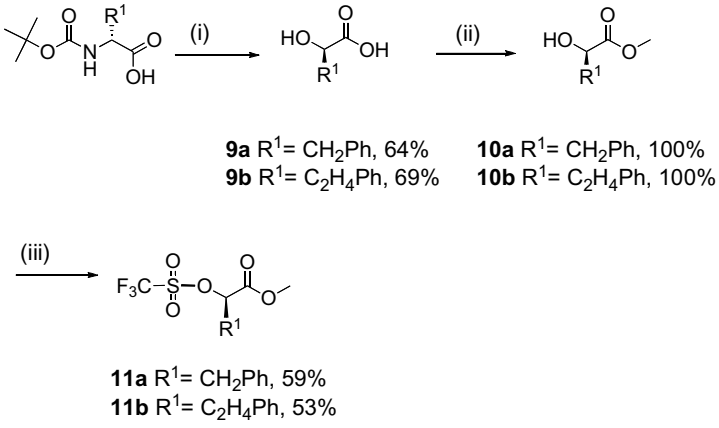
The triflates **11a–b** were then condensed with the pyridone fragment **8** to give intermediates **12a–b** (Scheme 3) in good yield. After purification on silica gel, the esters were quantitatively reduced to the primary alcohols **13a–b**, which were selectively oxidized to the corresponding aldehydes **5a–b**[27] (Scheme 3), via a Dess–Martin periodinane oxidation or, more successfully, using Swern conditions. Olefination of the aldehyde using Wittig– or Horner–Emmons–Wadsworth methodologies afforded target molecules **6a–c** (Scheme 3).[28] This is an approach widely applied to the synthesis of α,β unsaturated esters[29,30] and aryl or alkyl vinyl sulfones.[18,31,32]

The antimalarial activity of the target molecules prepared was measured in red blood cell-based assays. Efficacy was monitored by parasite {^{3}H}-hypoxanthine incorporation using parasite-infected human erythrocytes.[33,34] We have assayed compounds in triplicate against the chloroquine-resistant parasite strain TM6 and against the chloroquine-sensitive parasite strain 3D7 (Table 1). The screening of our three best peptidomimetics against purified recombinant FP2 and FP3 (prepared as previously described[8,10]) was performed as previously described by Shenai et al.[18]

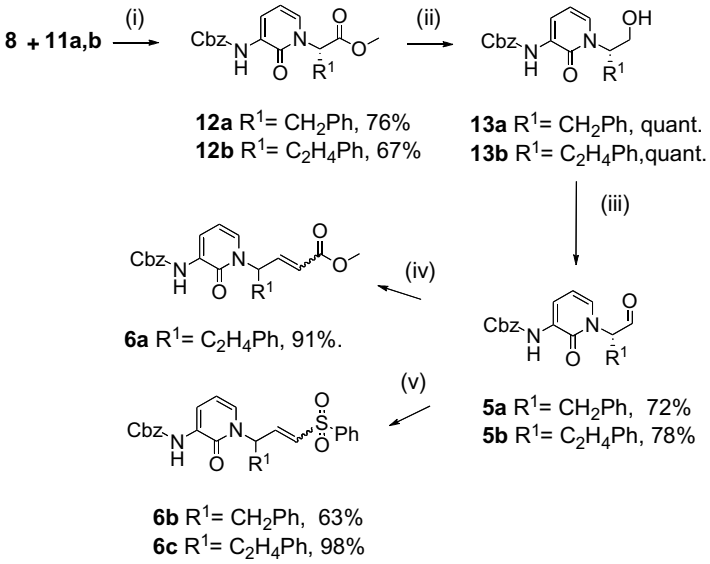
All our new peptidomimetic derivatives displayed measurable in vitro antimalarial inhibition, with IC_{50} ranging from 6 to 40 μM (initial synthetic approach with R^1 = afforded compounds with no measurable antimalarial activity). Compound **6c** was the best inhibitor of this series, followed by the α,β unsaturated methyl ester **6a**, with very close activity to the one presented by aldehyde **5b**. As seen previously, the vinyl sulfone **6b** and the aldehyde **5a**, bearing a phenylalanine substituent at P2 position, were less active than their counterparts bearing the longer unnatural segment of



Scheme 1. Reagents and conditions: (i) H_2, 10% Pd–C, EtOH, 8 h, rt.

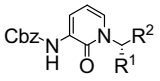


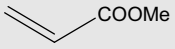
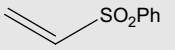
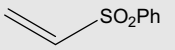
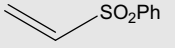
Scheme 2. Reagents and conditions: (i) HCl, dioxane, hexane, then H_2SO_4 and $NaNO_2$ aq; (ii) HCl, MeOH, overnight, rt; (iii) 2,6-lutidine, trifluoromethane sulfonic anhydride, neat, 0 °C, 1 h.



Scheme 3. Reagents and conditions: (i) NaH, THF, rt, 2 h; (ii) $NaBH_4$, $CaCl_2$, EtOH, −5 °C; (iii) a—DMP, CH_2Cl_2, 1 h, rt; b—DMSO, CH_2Cl_2, then $(COCl)_2$ and Et_3N; (iv) $Ph_3P{=}CO_2Me$, THF, reflux, 1 h; (v) NaH, diethyl(phenylsulfonyl)methylphosphonate, THF, −10 °C to rt, 1 h, 30 min.

Table 1
Antimalarial activities of peptidomimetic compounds ($R^1 \neq H$) versus *Plasmodium falciparum* parasites and recombinant enzymes FP2 and FP3



Compound	R^1	R^2	IC_{50}[a] (μM)			
			3D7[c] parasite strain	TM6[c] parasite strain	Rec FP2	Rec FP3
5a	CH_2Ph	CHO	37.3	36.0	—	—
5b	C_2H_4Ph	CHO	31.0	22.7	10.9	>25.0
6a(E:Z 9:1)	C_2H_4Ph		27.9	35.3	19.0	>25.0
6b (E)	CH_2Ph		35.7	36.0	—	—
6c (E:Z 1:1)	C_2H_4Ph		5.7	9.0	[b]	[b]
1a	C_2H_4Ph			0.003[d]	0.002[9]	0.021[9]
Chloroquine			15 nM	150 nM		

[a] Parasites were maintained in continuous culture, according to the method described by Trager and Jensen.[33] IC_{50} values were measured according to the method described by Desjardins et al.[34]
[b] Precipitation of the compound occurred in this case, not allowing reliable analysis.
[c] 3D7 is a chloroquine-sensitive strain of the parasite *P. falciparum*, and TM6 is a chloroquine-resistant strain of the parasite.
[d] Compound tested against Itg2 strain of *P. falciparum* parasites.[12]

homophenylalanine. These results are supportive with the fact that a longer chain is a more suitable substituent to have in the P1 recognition site of FP2 inhibitors. Though molecular modeling studies showed excellent match of conformations between both our peptidomimetic **6a** and **6c** and their peptidic counterparts, antimalarial activity is more significant with the vinyl sulfonyl analogue, indicating that this electrophilic moiety may be more reactive toward the nucleophilic thionyl group of the target enzyme. Also, we can see from our activity results that reversible aldehyde inhibitors show slightly higher activity against the chloroquine-resistant strain TM6. Activity of our three best peptidomimetics against purified recombinant enzymes FP2 and FP3 was found to be week (IC_{50} > 10 μM), with higher activity against FP2 than FP3, in line with previous reports of the selectivity of these types of compounds against parasite enzymes.[35] It is noteworthy that compound **5b** shows both higher antiplasmodial activity and enzymatic inhibition when compared to compound **6a**; however, the solubility problems encountered for compound **6c** under the experimental conditions used in this assay have not permitted, until the current time, to obtain a clear correlation between the antiparasitic and enzymatic inhibitory activities, which would strengthen the hypothesized mode of action for this class of compounds.

In summary, a new class of peptidomimetics have been synthesized, following a short and optimized synthetic route and incorporating a new pyridone ring scaffold as a replacement for the peptidic leucine residue, at the P2 position, within the inhibitor's framework. From analysis of the work described in this paper, it seems that though conformational restriction is reported to be a

requirement for inhibitors to be recognized by many proteases,[36,37] and despite our results being comparable and consistent to others for peptidomimetic cysteine protease inhibitors reported in the literature,[38] the rigid pyridine moiety chosen here is not a suitable backbone modification, as structurally comparable unconstrained peptidyl aldehydes and vinyl sulfones display higher activity.[5,12,18]

Acknowledgments

The authors thank the Biotechnology and Biological Sciences Research Council (BBSRC, S.A.W., P.O.N., P.G. Grant BB/C006321/1) and the FCT (Fundação para a Ciência e Tecnologia in Portugal, Ph.D. Grant: SFRH/6176/2001), and the National Institute of Health, Portugal for funding this work.

References and notes

1. Breman, J. G. *Am. J. Trop. Med. Hyg.* **2001**, *64*, 1.
2. Sajid, M.; McKerrow, J. H. *Mol. Biochem. Parasitol.* **2002**, *120*, 1.
3. Rosenthal, P. J.; McKerrow, J. H.; Aikawa, M.; Nagasawa, H.; Leech, J. H. *J. Clin. Invest.* **1998**, *82*, 1560.
4. Rosenthal, P. J.; Lee, J. K.; Smith, R. E. *J. Clin. Invest.* **1993**, *91*, 1052.
5. Olson, J. E.; Lee, G. K.; Semenov, A.; Rosenthal, P. J. *Bioorg. Med. Chem.* **1999**, *7*, 633.
6. Sijwali, P. S.; Koo, J.; Singh, N.; Rosenthal, P. J. *Mol. Biochem. Parasitol.* **2006**, *150*, 96.
7. Singh, N.; Sijwali, P. S.; Pandey, K. C.; Rosenthal, P. J. *Exp. Parasitol.* **2006**, *112*, 187.
8. Shenai, B. R.; Sijwali, P. S.; Singh, N.; Rosenthal, P. J. *J. Biol. Chem.* **2000**, *275*, 29000.
9. Dahl, E. L.; Rosenthal, P. J. *Mol. Biochem. Parasitol.* **2005**, *139*, 205.
10. Sijwali, P. S.; Shenai, B. R.; Gut, J.; Singh, N.; Rosenthal, P. J. *Biochem. J.* **2001**, *360*, 481.
11. Rosenthal, P. J.; Wollish, W. S.; Palmer, J. T.; Rasnick, D. *J. Clin. Invest.* **1991**, *88*, 1467.
12. Rosenthal, P. J.; Olson, J. E.; Lee, G. K.; Palmer, J. T.; Klaus, J. L., et al *Antimicrob. Agents Chemother.* **1996**, *40*, 1600.
13. Hans-Haartwig, O.; Schirmester, T. *Chem. Rev.* **1997**, *97*, 133.
14. Powers, J. C.; Asgian, J. L.; Ozlem, D. E.; James, E. K. *Chem. Rev.* **2002**, *102*, 4639.
15. Lecaille, F.; Kaleta, J.; Bromme, D. *Chem. Rev.* **2002**, *102*, 4459.
16. Lee, B. J.; Singh, N.; Chiang, P.; Kemp, S. J.; Goldman, E. A., et al *Antimicrob. Agents Chemother.* **2003**, *47*, 3810.
17. Cheng, H. *J. Org. Chem.* **1994**, *59*, 7671.
18. Shenai, R. B.; Lee, B. J.; Alvarez-Hernandez, A.; Chong, P. Y.; Emal, C. D., et al *Antimicrob. Agents Chemother.* **2003**, *47*, 154.
19. Scheidt, K. A.; Roush, W. R.; McKerrow, J. H.; Selzer, P. M.; Hansell, E., et al *Bioorg. Med. Chem.* **1998**, *6*, 2477.
20. Leung-Tong, R.; Li, W.; Tam, T. F.; Karimian, K. *Curr. Med. Chem.* **2002**, *9*, 979.
21. Zhu, S.; Hudson, T. H.; Kyle, D. E.; Lin, A. J. *J. Med. Chem.* **2002**, *45*, 3491.
22. Dolle, R. C.; Prouty, C. P.; Cook, E.; Saha, A.; Ross, T. M., et al *J. Med. Chem.* **1996**, *39*, 2438.
23. Dragovich, P. S.; Prins, T. J.; Zhou, R.; Brown, E. L.; Maldonado, F. C., et al *J. Med. Chem.* **2002**, *45*, 1607.
24. FieldTemplater 2.0.1, http://www.cresset-bmd.com.
25. DeLano, W. L. The PyMOL Molecular Graphics System DeLano Scientific, 2002, http://www.pymol.org. Palo Alto, CA, USA.
26. Warner, P.; Green, R. C.; Gomes, B.; Strimpler, A. M. *J. Med. Chem.* **1994**, *37*, 3090.
27. *Procedure for the synthesis of peptidomimetic pyridone aldehydes* **5a,b**: DMSO (3.02 mmol) was added to a solution of oxalyl chloride (1.81 mmol) in DCM (0.55 ml) at −78 °C, and stirred for 5 min. The alcohol (1.01 mmol) dissolved in DCM (15 ml) was then added, and the mixture was stirred for 15 min still at −78 °C, after which triethylamine (7.05 mmol) was added. The reaction mixture was allowed to warm up to 0 °C and kept at that temperature until TLC analysis indicated consumption of the starting material. The reaction mixture was then diluted with water, washed with brine and saturated aq $NaHCO_3$. The organic layer was dried over $MgSO_4$, evaporated and purified by flash column chromatography. *Procedure for the synthesis of compound* **5a**: This product was prepared in 72% as a clear yellow oil. The product was purified by flash column chromatography using 45% EtOAc in hexane as the eluent system. $[\alpha]_D^{22}$ −55° (*c* 0.4, CH_2Cl_2). ν_{max} (neat)/cm^{-1} 3374, 3030, 2924, 1730, 1646, 1594, 1560, 1512, 1454, 1381, 1359, 1260, 1199, 1163, 1071, 1029, 917, 744, 699. ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.98 (d, *J* = 6.4 Hz, 1H), 7.89 (s, 1H), 7.40–7.10 (m, 10H, Ar), 6.87 ((dd, *J* = 6.8 Hz, *J* = 1.2 Hz, 1H), 6.15 (t, *J* = 7.2 Hz, 1H), 5.18 (s, 2H), 4.90 (br s, 1H), 3.92 (br s, CHO), 3.16 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.1, 153.7, 137.4, 136.4, 129.8, 129.1, 129.0, 128.7, 128.5, 128.3, 127.3, 120.5, 107.1, 67.4, 63.9. MS found $[M+Na^+CH_3OH]^+$ 431.1577, $C_{23}H_{24}N_2O_5{}^{23}Na$ requires 431.1583. *Procedure for the synthesis of compound* **5b**: this product was prepared in 78% as a yellow oil. The product was purified by flash column chromatography using 45% EtOAc in hexane as the eluent system. $[\alpha]_D^{22}$ −184° (*c* 0.8, CH_2Cl_2). ν_{max} (neat)/cm^{-1} 3386, 1732, 1649, 1597, 1564, 1512, 1454, 1381, 1360, 1261, 1199, 1160, 1066. ^1H NMR (400 MHz, $CDCl_3$) δ_H 9.60 (s, 1H, CHO), 8.08 (d, *J* = 7.2 Hz, 1H), 7.82 (s, 1H), 7.40–7.14 (m, 10H, Ar), 6.75 (dd, *J* = 7.2 Hz, *J* = 1.6 Hz, 1H), 6.30 (t, *J* = 7.2 Hz, 1H), 5.21 (s, 2H), 4.75 (dd, *J* = 9.6 Hz, *J* = 4.0 Hz, 1H), 2.70–2.58 (m, 3H), 2.34–2.22 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 195.7, 157.1, 153.3, 139.7, 135.9, 129.9, 128.7, 128.6,128.4, 128.1, 128.1, 126.6, 120.5, 107.3, 67.4, 67.2, 29.7, 29.4, 30,0. MS found $[M+H]^+$ 391.1663, $C_{23}H_{23}N_2O_4$ requires 391.1658.
28. *Procedure for the synthesis of peptidomimetic pyridone vinyl ester* **6a**: Precursor aldehyde (0.59 mmol) was dissolved in dry tetrahydrofuran (8.7 ml), and (methoxycarbonylmethylene)–triphenylphosphorane (0.83 mmol) added. The reaction mixture was refluxed for 1 h then left stirring at room temperature, overnight. The crude mixture obtained was purified by flash chromatography, using 40% EtOAc in hexane as the eluent system, affording the pure product as a viscous yellow oil (91%). ν_{max} (neat)/cm^{-1} 3377, 1728, 1649, 1601, 1512, 1454, 1437, 1389, 1356, 1263, 1198, 1066. ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.02 (d, *J* = 7.2 Hz, 1H), 7.91 (s, 1H, NH), 7.41–7.10 (m, 10H, Ar), 6.99 (dd, *J* = 15.6 Hz, *J* = 5.2 Hz, 1H, vinyl H), 6.85 (dd, *J* = 7.2 Hz, *J* = 2.0 Hz, 1H), 6.29 (t, *J* = 7.2 Hz, 1H), 5.82 (dd, *J* = 15.6 Hz, *J* = 2.0 Hz, 1H, vinyl H), 5.77 (m, 1H), 5.21 (s, 2H), 3.72 (s, 3H), 2.68–2.60 (m, 1H), 2.56–2.49 (m, 1H), 2.29–2.12 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.0, 157.1, 153.4, 145.1, 140.1, 136.0, 129.6, 128.6, 128.3, 128.3, 128.1, 126.4, 125.2, 123.2, 119.5, 107.3, 67.1, 55.8, 51.8, 34.7, 32.0, 30.9. MS found $[M+Na]^+$ 469.1722, $C_{26}H_{26}N_2O_5{}^{23}Na$ requires 469.1739. *Procedure for the synthesis of peptidomimetic pyridone vinyl phenyl sulfones* **6b,c**: To a solution of diethyl (phenylsulfonyl)methyl phosphonate previously prepared (0.30 mmol) in THF (3 ml), at −10 °C, was added sodium hydride (0.32 mmol). After gas evolution had ceased (approx. 30 min), a solution of the aldehyde precursor (0.24 mmol) in THF (3 ml), at 0 °C, was added under nitrogen and with stirring. The reaction mixture was allowed to warm to 25 °C, with continuous stirring. After 1 h 30 min the reaction mixture was diluted with diethyl ether (15 ml) and then poured into brine (10 ml). The layers were separated, and the organic portion was dried over magnesium sulphate, filtered, and the filtrate concentrated under reduced pressure. The products were purified by flash column chromatography, using a 35% EtOAc in hexane solvent system. Compound **6b** was obtained as a pink oil (63%, analysis shown for the E product): $[\alpha]_D^{22}$ −32° (*c* 1.0, CH_2Cl_2). ν_{max} (neat)/cm^{-1} 3377, 3063, 2925, 1729, 1648, 1599, 1559, 1507, 1447, 1387, 1357, 1306, 1198, 1148, 1086, 1069, 745, 688. ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.86 (m, 2H), 7.73–6.98 (m, 16H), 6.74 (dd, *J* = 7.2 Hz, *J* = 1.6 Hz, 1H), 6.18 (dd, *J* = 15.0 Hz, *J* = 2.0 Hz, 1H, vinyl), 6.15 (t, *J* = 7.2 Hz, 1H), 5.90–5.85 (m, 1H), 5.10 (s, 2H), 3.17–3.03 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 157.1, 153.6, 140.0, 134.0, 133.5, 129.9-127.7, 125.84, 120.0, 107.6, 73.3, 68.4, 67.5, 61.6, 57.6, 53.8, 44.8, 39.6, 32.0, 30.1, 25.7. Found $[M+Na]^+$ 537.1453, $C_{29}H_{26}N_2O_5S^{23}Na$ requires 537.1460 and $[M+K]^+$ 553.1220, $C_{29}H_{26}N_2O_5S^{39}K$ requires 553.1200. Compound **6c** was obtained as a colorless oil (98%, analysis shown for the E/Z product mixture): $[\alpha]_D^{22}$ −137° (*c* 0.3, CH_2Cl_2). ν_{max} (neat)/cm^{-1} 3375, 1728, 1649, 1604, 1512, 1504, 1446, 1358, 1308, 1252, 1199, 1145, 1084, 902., 872. ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.05–7.01 (m), 6.66 (dd, *J* = 7.2 Hz, *J* = 2.0 Hz), 6.36 (dd′, *J* = 6.8 Hz, *J* = 2.0 Hz), 6.19 (t, *J* = 7.2 Hz), 6.12 (t′, *J* = 6.8 Hz), 5.73 (dd′, *J* = 10.8 Hz, *J* = 5.2 Hz), 5.55 (t, *J* = 8.4 Hz), 5.21 (s, 2H), 5.20 (s′, 2H), 3.83 (d, *J* = 8.4 Hz, 2H), 3.66 (m, 2H), 3.48 (dd′, *J* = 14.4 Hz, *J* = 10.8 Hz), 3.05 (s), 2.80–2.72 (m), 2.67 (t, *J* = 8.4 Hz, 2H), 2.43 (t′, *J* = 7.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$)δ_C 156.3, 155.8, 153.3, 153.2, 149.3, 146.7, 139.8, 139.7, 138.6, 138.6, 135.9, 135.8, 134.1, 133.9, 133.6, 129.5, 129.3, 128.6, 128.5, 128.3, 128.2, 128.1, 127.3, 126.4, 120.2, 120.1, 116.9, 114.7, 106.5, 106.5, 67.1, 55.3, 55.1, 44.4, 36.1, 32.4, 32.40, 31.6, 30.6, 25.3. Found $[M+H]^+$ 551.1601, $C_{30}H_{28}N_2SO_5$ ^{23}Na requires 551.1617.
29. Dragovich, P. S.; Webber, S. E.; Binford, S. L.; Babine, R. E.; Lee, B. J., et al *J. Med. Chem.* **1998**, *41*, 2806.
30. Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405.
31. Enders, D.; Von Berg, S.; Jendeleit, B. *Org. Synt.* **1995**, *78*, 169.
32. Carretero, J. C.; Demillequand, M.; Ghosez, L. *Tetrahedron* **1987**, *43*, 5125.
33. Trager, W.; Jensen, J. B. *Science* **1976**, *193*.
34. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710.
35. Sijwali, P. S.; Kato, K.; Seydel, K. B.; Gut, J.; Lehman, J., et al *Proc. Natl. Acad. Sci. U.S.A.* **2004**, 8721.
36. Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305.
37. Salituro, F. G.; Baker, C. T.; Court, J. J.; Deininger, D. D.; Kim, E. E., et al *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3637.
38. Ettari, R.; Nizi, E.; Di Francesco, M. E.; Dude, M. A.; Pradel, G., et al *J. Med. Chem.* **2008**, *51*, 996.