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Design and Synthesis of Conformationally Restricted Eight-Membered Ring Diketones as Potential Serine Protease Inhibitors

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Abstract—The design of conformationally restricted eight-membered ring diketones as transition state mimics of the mechanism of action of cyclotheonamides on serine proteases is described. Two target compounds are prepared from mutilin, derived from the natural product pleuromutilin. Compound **3** shows significant inhibition of plasmin and urokinase in enzyme rate assays, but an analogue **4** in which the amide moiety has been omitted does not. An X-ray crystal structure of the diketone **3** confirms the conformational predictions made by molecular modelling.

Cyclotheonamide A[1] (CyA; **1**) is a macrocyclic pentapeptide with potent serine protease inhibitor activity. Several structural variants of CyA are also known to possess this activity and this has allowed some SAR to be observed.[1–4] Central to the mechanism of action of these compounds is interaction of the α-ketoamide moiety with the serine and histidine residues of the catalytic triad as shown by crystal structure analysis of CyA bound to α-thrombin[5] and trypsin.[6] In the complex with thrombin, the α-keto amide group of CyA is involved in a tetrahedral intermediate hemi-ketal structure with Ser^{195} at the active site. Furthermore, the keto oxygen makes a bifurcated hydrogen bond with the imidazole residue of the active site His^{57}. In common with many other productive binding substrates and inhibitors of thrombin and trypsin, CyA possesses an arginine residue which is known to occupy the S1 specificity pocket. Many serine proteases are known to adopt the same conformation around the active site such that changing the specificity residues on a common inhibitor backbone to those appropriate to a particular protease will produce inhibitors specific to that protease.[7] We were intrigued by the consideration that it might be possible to construct a generic small molecule serine protease inhibitor that retains key features of CyA. Enhancements in potency and/or selectivity could be achieved by subsequent introduction of recognition elements appropriate for S1 and other specificity pockets. In order to achieve this, the skeleton must conformationally restrict the α-ketoamide moiety such that the two carbonyl groups are orientated at a dihedral angle consistent with that suggested by the X-ray structures of the inhibitor/protease complexes. Additionally, the S1 specificity residue must be suitably spacially orientated relative to the α-ketoamide.

Other studies had given us considerable knowledge of the conformation of the eight-membered ring in the Mutilin nucleus **2**.[8] We surmised that conversion of **2** to the α-diketone **3** would result in the desired dihedral angle between these two carbonyl groups allowing this portion of the molecule to act as an α-ketoamide surrogate. In addition, the C6 methyl group would be orientated appropriately to fill an S1 recognition pocket. The vinyl group in **2** could also be converted into an amine or amide to allow mimicry of the hydrogen bond of the N7 hydrogen atom (numbering as shown in Fig. 1) to the Ser^{214} carbonyl oxygen atom (α-thrombin numbering).[5] Compound **3** was selected as a probe to explore the validity of this concept with the intention to design and synthesise further conformationally restricted eight-membered α-ketoamide ring systems.

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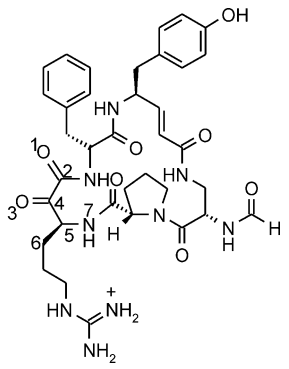


Figure 1. Structure of cyclotheonamide A; numbering shows atoms used for r.m.s.d. fitting.

We now present results of the molecular modelling, synthesis, and X-ray crystal structure studies of mutilin derivatives (e.g., **3**) and protease inhibition results against a range of proteases.

The molecular modelling procedures used in this study began with an examination of the low energy conformers of **3**. Conformers were generated using DGEOM and minimised using CHARMM (conjugate gradient method). Five conformers were found within a 5 kcal range of the lowest energy conformer, indicating limited flexibility around the dicarbonyl unit, amide, six-membered ring and the dimethylene unit in the eight-membered ring. These conformers were compared with the X-ray crystal structure of cyclotheonamide A bound to thrombin.[6] The lowest energy conformer showed a seven atom r.m.s.d. of 0.13 Å (see Figs. 1 and 2 for atoms used in the fitting procedure). The amide NH hydrogen atom in **3** was also sub-optimally orientated due to steric hindrance by the eight-membered ring. Diketone **4** gave similar results with increased flexibility about the dimethylene unit in the eight-membered ring. Based on modelling studies, it was decided to prepare diketones **3** and **4** as test cases for the general proposal that conformationally restricted eight-membered rings containing 1,2-dicarbonyls provide a scaffold suitable for serine protease inhibition.

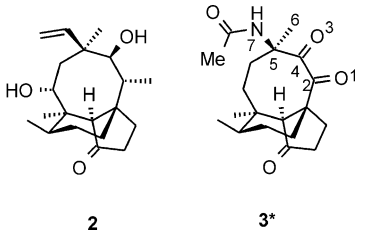
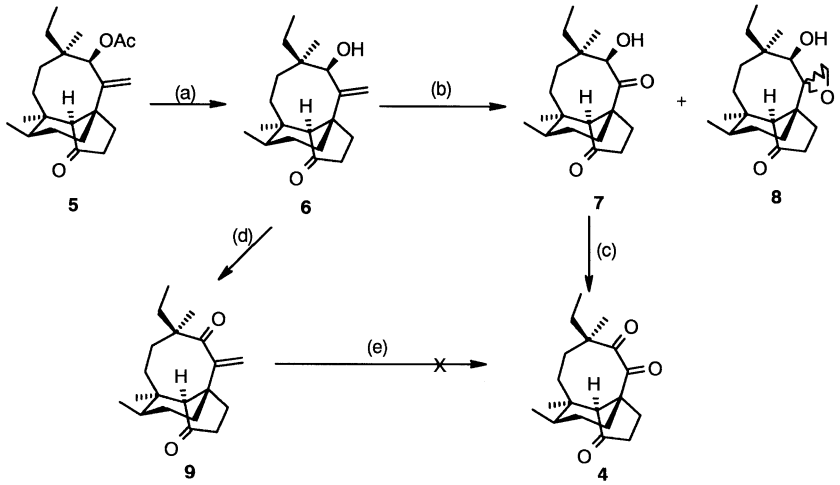


Figure 2. Structure of mutilin **2** and proposed serine protease inhibitor **3**. Numbering indicates atoms used in r.m.s.d. fitting procedures.

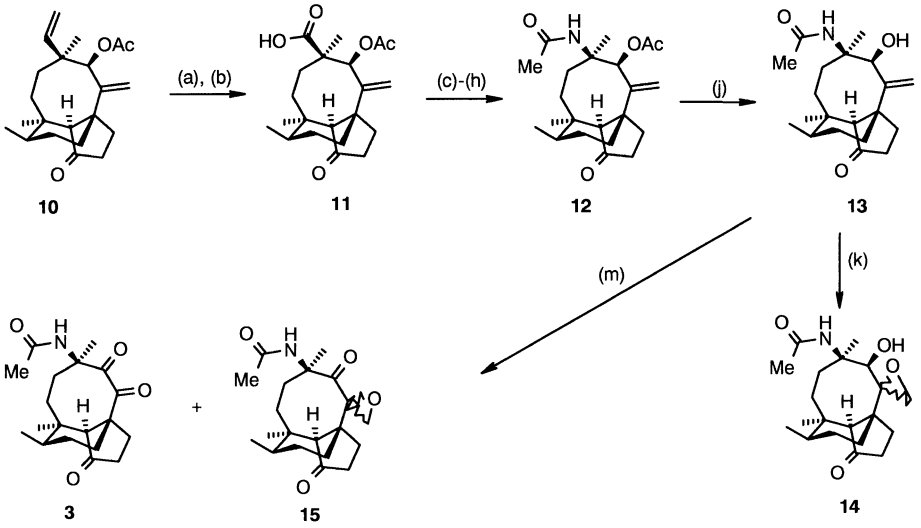
In order to develop suitable methodology the less functionalised molecule **4** was prepared first (Scheme 1). The acetate **5**[9] was hydrolysed to the alcohol **6** that, upon ozonolysis in methanol, gave only the epoxides **8**. However, switching the solvent to toluene produced, in addition to **8**, the required hydroxyketone **7** in low yield. A range of oxidising agents were investigated for conversion of the alcohol **7** to the diketone **4**, with the best results being achieved with DMSO/acetic anhydride, to produce diketone **4** as a gum in 16% yield.[10] The alternative route of oxidation to the keto-olefin **9** and subsequent ozonolysis was not successful.

The synthesis of **3** was then initiated (Scheme 2). Acetate **10**, obtained analogously to **5** from mutilin **2**,[9] was oxidised in a stepwise fashion via aldehyde to the acid **11**. Sequential acyl azide formation, Curtius rearrangement, hydrolysis, and acetylation produced the required amide **12** in 36% overall yield. Base hydrolysis to the alcohol **13** and subsequent ozonolysis in methanol or toluene only produced the epoxides **14**. However, oxidation with sodium periodate and ruthenium trichloride,[11] and purification by column chromatography, produced the required solid diketone **3** along with epoxides **14** and the keto-epoxides **15**.[12]

The X-ray crystal structure of **3** (Fig. 3) shows a seven atom r.m.s.d. of 0.07 Å to the lowest energy conformer generated by molecular modelling (see Fig. 2 for atoms used in the fitting procedure). Inspection of the B factors showed that the greatest flexibility was around the amide unit and six-membered ring as suggested by the



Scheme 1. Preparation of diketone **4**. (a) NaOH, dioxan, 60%; (b) ozone, toluene; 16% of **7**; (c) DMSO, Ac_2O, 13%; (d) DMSO, Ac_2O, 16%; (e) ozone, toluene.



Scheme 2. Preparation of diketone **3**. (a) $NaIO_4$, OsO_4 (cat.), THF, H_2O, 78%; (b) Jones oxidation, 75%; (c) $(COCl)_2$, DMF, CH_2Cl_2; (d) NaN_3; (e) benzene, heat; (f) HCl; (h) Ac_2O, 36% for (c) to (h); (j) NaOH, dioxan, 72%; (k) ozone, toluene or methanol; (m) $NaIO_4$, $RuCl_3$, CCl_4, MeCN, H_2O, 17% of **3**.

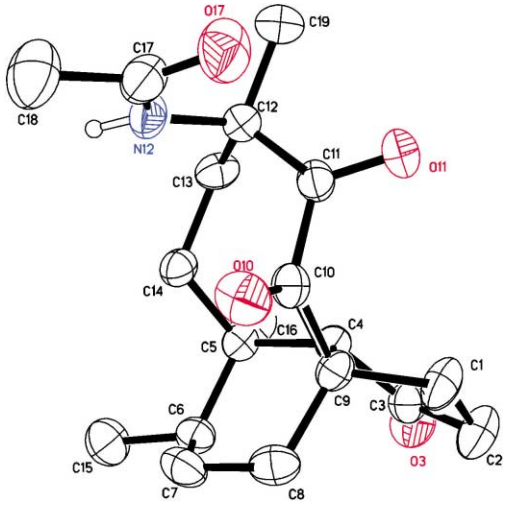


Figure 3. A view of **3**[11,14] from its crystal structure showing the numbering scheme employed. Hydrogen atoms are displayed with an arbitrarily small radius. Thermal displacement ellipsoids for non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms from methyl groups have been omitted for clarity.

modelling study. Unfortunately, in the solid state structure, the amide NH is not optimally orientated for interaction with a serine protease based upon previous observations in protein–CyA complexes.[7] However, the flexibility around the exocyclic amide to ring bond seen in the crystal structure may allow the side chain-amide NH to adopt a conformation that enables a sub-optimal H-bond to the protease backbone.

Diketones **3** and **4** were assayed for protease activity[13] using high-throughput enzyme rate assays employing chromogenic tri-peptide substrates, under conditions where there was no indication of substrate depletion. This suggests that initial kinetic rates were being observed. At 100 μM, **4** failed to show any significant inhibition. Gratifyingly, the amide derivative **3**, which by virtue of the amide NH is expected to form an additional key protease interaction,[7] shows modest inhibition of plasmin and urokinase (25 and 28%, respectively). A methyl substituent in the S1 pocket is considered to be less than ideal for both of these proteases and it would be predicted that optimal substituents would produce considerably more potent inhibitors.

In conclusion, in the present study we have demonstrated that a conformationally restricted eight-membered ring dicarbonyl system can serve as a template for serine protease inhibitors and anticipate that further modifications of this structural class may lead to molecules exhibiting enhanced activity.

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37.6, 52.6, 53.0, 57.7, 208.1, 213.5 and 213.8. IR ($CHCl_3$) 1689, 1701 and 1741 cm^{-1}. MS *m*/*z* 304 (*M*, C_{19} H_{28} O_3).
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12. Data for **3**; mp 280 °C (from CH_2Cl_2–hexane), ^{13}C NMR (400 MHz), δ 15.0, 22.0, 22.1, 23.2, 25.8, 26.0, 26.5, 26.5, 28.8, 29.7, 30.2, 34.9, 35.8, 37.4, 53.0, 58.0, 64.7, 170.5, 205.0, 205.2, and 213.6. IR (KBr) 1519, 1679, 1706 and 1735 cm^{-1}. MS *m*/*z* 334 (*M*, C_{19} H_{27} N O_4). Anal. calcd for (C_{19} H_{27} N O_4): C, 68.44; H, 8.16; N, 4.20. Found: C, 68.37; H, 7.94; N 4.34%.
13. Enzyme inhibition against a range of proteases including plasmin and urokinase was measured using a chromogenic tripeptide substrate assay. The substrate concentration was 500 mM and enzymes were diluted so that linear kinetics were observed; inhibitor concentrations were typically at a 1000–2000 molar excess over enzyme. Activity rates were determined using Reader Manager Software calculations based on total OD change/time.
14. Crystallographic data (excluding structure factors) for **3** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. 134704. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).