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# Solid-Phase Synthesis of Cyclic Alkoxyketones, Inhibitors of the Cysteine Protease Cathepsin K

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**Abstract**—Using solid-phase synthesis, a library of novel cyclic alkoxyketones has been constructed which show strong inhibitory activity against the cysteine protease, cathepsin K (EC 3.4.22.38). © 2001 Published by Elsevier Science Ltd.

The realisation that many members of the cysteine protease family are selectively expressed in specialised tissues and have high sequence specificity has led to their attractiveness as therapeutic targets (e.g. arthritis, cancer, parasitic and viral diseases).[1] Cathepsin K (EC 3.4.22.38), a cysteine protease of the papain superfamily, is selectively expressed in osteoclasts and has been implicated in the process of bone resorption.[2] Selective inhibitors of cathepsin K therefore could be promising therapeutic agents for the treatment of diseases characterised by excessive bone loss, such as osteoporosis.

Initial work from our co-workers[3] had identified a series of acylic alkoxymethylketones, typified by analogue **1**, as potent cathepsin K inhibitors. Important to the design of these analogues was an α-heteroatom which served to increase the reactive nature of the carbonyl moiety towards formation of a tetrahedral intermediate with Cys25 in the active site. In an effort to improve the in vitro and in vivo properties of these acyclic ketones, compounds involving cyclisation from the amino acid alpha to the keto moiety onto the alkoxymethyl group to afford a series of cyclic alkoxyketones (Fig. 1) were synthesised.[4] Although our initial analogue **2** displayed a 2-fold reduction in potency relative to the acyclic analogue **1**, albeit for a mixture of two diastereomers, we decided to explore further the SAR in this novel series based around template **3**. Herein we report a highly efficient solid-phase synthesis of cyclic alkoxyketones **3** and the initial SAR to emerge from this study.

Our retrosynthetic approach to the combinatorial synthesis of this class of inhibitor evolved from the work of Barany,[5] in which we envisioned attachment of our inhibitors via a reductive amination of an amino acid with a 3,5-dimethoxybenzyl linker **4** (BAL) (Scheme 1). Subsequent manipulation would then afford our resin bound inhibitors **5** which, when treated with acid, would lead to resin cleavage, with concomitant removal of the ketal protecting group, affording inhibitors **3**.

Thus, reductive amination of 10 equiv $H_2NCHR^2CO_2Me$ with the resin-bound aldehyde **4** using 10 equiv $NaBH(OAc)_3$ in DMF provided the secondary amine **6**, which was acylated by treatment with 3 equiv of $R^1CO_2H$, 3 equiv EDC in DMF at room temperature for 12 h. Hydrolysis of ester **7** with aqueous base led to racemisation of the chiral centre. This racemisation could be avoided by treatment of ester **7** with 10 equiv potassium trimethylsilanoate in DCM at room temperature for 12 h. Treatment of resulting acid **8** with 3 equiv of amine **9**[6] and 3 equiv of EDC in DMF at room temperature for 3 h afforded the resin bound inhibitor **5** protected as the dimethyl ketal. Finally, cleavage from the resin with concomitant deprotection of the dimethylketal was accomplished with TFA:DCM:$H_2O$ (7:2:1) affording the desired cyclic alkoxy ketone products **1** as a mixture of two diastereomers, in good yield and purity[7] (Scheme 2).

The synthesis of arrays utilising this synthetic protocol could also be carried out employing ACT496 robotics or IRORI radio frequency encoded combinatorial chemistry technology (REC).[8] Utilising the latter technology, multi-milligram quantities of discrete analogues could

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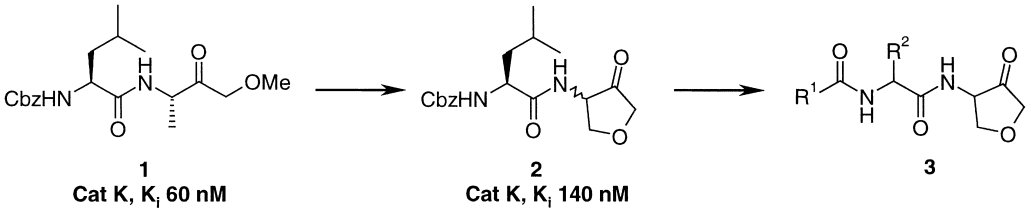
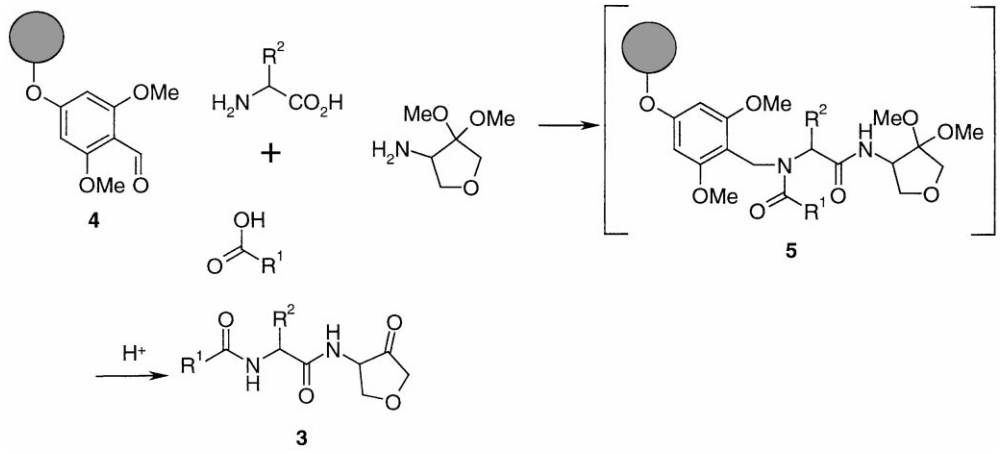
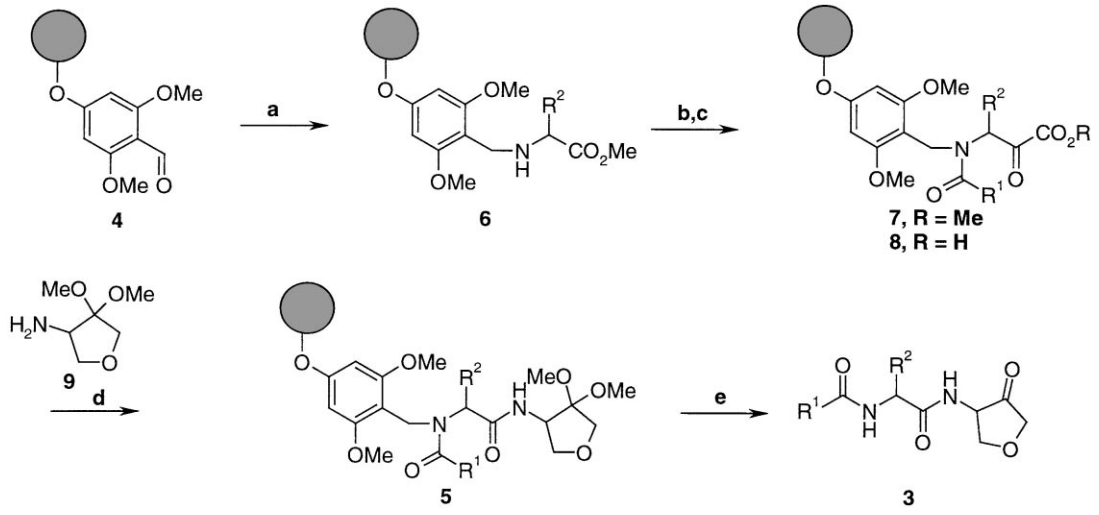



**Figure 1.** The evolution of cyclic alkoxyketones.




**Scheme 1.** Combinatorial approach to cyclic alkoxyketones.




**Scheme 2.** Solid phases synthesis of cyclic alkoxyketones. Conditions: (a) $H_2NCHR^2CO_2Me$, $Na(OAc)_3BH$, DMF; (b) $R^1CO_2H$, EDC, DMF; (c) KOSiMe, DCM; (d) **9**, EDC, DMF; (e) TFA/DCM/$H_2O$ (7:2:1).

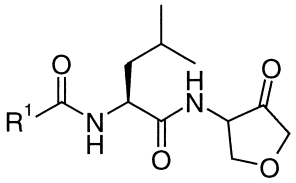
be prepared exploiting the more efficient split/pool technique.[9]

The solid-phase synthesis of the above class of inhibitors allowed a wide range of $R^1$ and $R^2$ substituents to be explored simultaneously. For conciseness, however, the $R^1$ and $R^2$ SAR has been summarised to focus on those substituents which led to a dramatic modulation of cathepsin K inhibition.

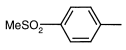
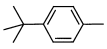
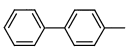
The size, shape and electronic properties of the $R^1$ group were varied in order to probe the cathepsin K enzyme, and these results are summarised in Table 1.

No enzyme inhibition was seen with a number of non-aromatic analogues such as **10a** and **10b**. Inhibitors containing a lipophilic aromatic $R^1$ substituent (**10e** and **10f**) led to excellent inhibition of cathepsin K. Introduction of electron withdrawing groups (**10g**, **10h** and **10j**) gave a significant reduction in potency relative to the parent analogue **10c**. Interestingly, a number of potent heterocyclic substituents were identified with the benzo[*b*]thiophene analogue **10o** demonstrating a 60-fold increase over its related thiophenyl analogue **10n**. The above SAR is consistent with a number of X-ray co-crystal structures of inhibitors bound within the cathepsin K active site. In these examples important π–π

**Table 1.** Representative members of the cyclic alkoxylketone libraries with $K_i$ (nM) shown against human cathepsin K



**10**

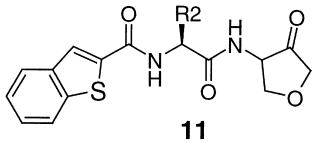
| Compound | $R^1$ | $K_i$ (nM)[10] | Compound | $R^1$ | $K_i$ (nM) |
|---|---|---|---|---|---|
| **10a** | $CH_3$ | >1000 | **10j** |  | 710 |
| **10b** |  | >1000 | **10k** |  | 420 |
| **10c** |  | 180 | **10l** |  | 44 |
| **10d** |  | 64 | **10m** |  | 61 |
| **10e** |  | 15 | **10n** |  | 640 |
| **10f** |  | 19 | **10o** |  | 11 |
| **10g** |  | >1000 | **10p** |  | 53 |
| **10h** |  | 820 | **10q** |  | 38 |

aromatic interactions between the bound ligand and either Tyr67 in the P3 pocket and/or Trp184 in the P3′ pocket have been postulated.[3,11]

Having identified the benzo[*b*]thiophene **10o** as our best $R^1$ substituent, Table 2 summarises some of our findings for the $R^2$ substituent with our optimal $R^1$ substituent.

Interestingly, the leucyl analogue **10o** was confirmed as our optimal $R^2$ substituent while the close analogue **11d** showed a 20-fold drop in potency, indicating cathepsin K displays high substrate specificity.

**Table 2.** Variation of the $R^2$ substituent with $K_i$ (nM) shown against human cathepsin K



**11**

| Compound | $R^2$ | $K_i$ (nM)[10] | Compound | $R^2$ | $K_i$ (nM) |
|---|---|---|---|---|---|
| **11a** | H | >10,000 | **11f** | $CH_2$-cyclohexyl | >10,000 |
| **11b** | Me | >10,000 | **11g** | $CH_2$-imidazoyl | >10,000 |
| **11c** | *i*-Pr | >10,000 | **11h** | $(CH_2)_4NH_2$ | >10,000 |
| **11d** | Pr | 190 | **11j** | $(CH_2)_2CONH_2$ | >10,000 |
| **10o** | *i*-Bu | 11 | **11k** | $(CH_2)_2CO_2H$ | >10,000 |
| **11e** | Bn | >10,000 | **11l** | $CHCH_3OH$ | >10,000 |

In summary, we have generated a library of reversible[12] cathepsin K inhibitors based around a novel cyclic alkoxyketone template by means of an efficient route employing solid-phase synthesis enabling the rapid identification of a number of potent cathepsin K inihibitors.

The vast therapeutic potential postulated for cysteine protease inhibitors also makes this library extremely valuable in the identification of potent inhibitors of other cysteine proteases. Further studies on this novel class of cysteine protease inhibitor will be reported in due course.

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12. Incubation of **2** with cathepsin K for 2 h followed by LC-MS analysis of the protein/inhibitor mixture showed no covalent modification of the protein. Pre-incubation of excess of **2** with cathepsin K for 2 h followed by addition of the fluorescent substrate Z-Phe-Arg-AMC revealed no loss of enzyme activity.