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5,5-Fused thiophene γ-lactams as templates for serine protease inhibition

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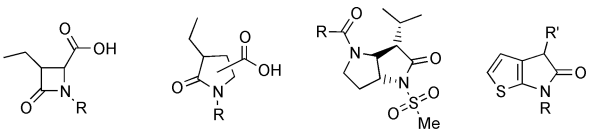
Novel 5,5-fused thiophene lactams are potent inhibitors and acylating agents of HNE and PPE.

Human neutrophil elastase (HNE) (EC 3.4.21.37) is a serine protease of the chymotrypsin family that has been implicated in the development of diseases including emphysema, cystic fibrosis, and rheumatoid arthritis.[1] Amongst known inhibitors of HNE are acylating agents, including both monocyclic (**1**) and bicyclic β-lactams.[2] Most of these lactams form stable acyl-enzyme complexes by reacting with the nucleophilic serine-195 of elastase.[3–5] X-Ray crystallographic analyses of a β-lactam (**1**) acyl-enzyme complex have shown that its inhibitory potency



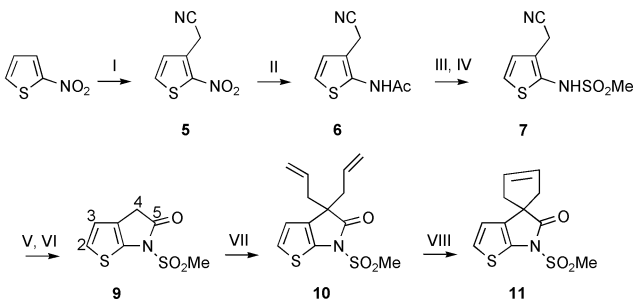
was not only due to irreversible acylation, but also to the hydrolytic stability of the newly formed ester link.[5] An important factor in stabilising the acyl-enzyme complex was rotation of the ester carbonyl out of the oxyanion hole. In contrast, analogous monocyclic γ-lactams (**2**), are relatively weak inhibitors of elastases.[6] In one such series, this is due, in part at least, to reversible formation of the γ-lactam from the acyl-enzyme and in another series also due to the enhanced hydrolytic lability of the acyl-enzyme in which the ester carbonyl is located in the oxyanion hole. Thus, hydrolytically stable acyl-enzyme complexes are only formed when the hydrolytic water molecule itself is displaced or another aspect of the catalytic machinery is perturbed.[7]

In contrast 5,5-*trans*-fused γ-lactams[8] such as (**3**) appear to react irreversibly with HNE and other serine proteases and are potent inhibitors with IC_{50} values of < 0.05 μM being observed in some cases.[9] Compared to monocyclic γ-lactams, the synthesis of the 5,5-*trans*-lactams is relatively complex. We are interested in providing cost-effective generic templates for protease inhibition. The choice of target templates took into account initial recognition, the likelihood of acyl-enzyme complex formation and its stability with respect to hydrolysis as well as the ease of synthesis and a minimisation of the number of chiral centres. Here we report research towards serine protease inhibitors based upon fused thiophene γ-lactams (**4**) synthesised with the aim of combining the potency of the *trans*-fused pyrrolidino-γ-lactams (**3**) and the ease of preparation of the monocyclic γ-lactams. It was hoped that the thiophene ring system, during inhibition, would stabilise the acyl-enzyme complex with respect to reformation of the γ-lactam ring, due to resonance with the amine lone pair released upon ring opening. The choice of methanesulfonyl and alkyl groups for derivatisation of the lactam nitrogen and the position α- to the lactam carbonyl was based upon previous studies with elastase.

t-BuOK mediated alkylation of 2-nitrothiophene with $ClCH_2CN$[10] was followed by reductive acetylation of the 2-nitro group.[11] Methanesulfonylation of the acetamido group was problematic with standard procedures,[6] but was successfully achieved using the Rapoport method.[12] Hydrolysis of the nitrile to the carboxylic acid followed by lactamisation[13] yielded the sulfamoylated lactam (**9**) (C=O stretch, 1732 cm^{-1}). Efficient base mediated (KHMDS or *t*-BuLi) alkylation of (**9**) to give mono-alkylated products (*e.g.* with EtI, MeI or EtOTf) was not achieved, but di-alkylated products such as bis-allylated thiophene-lactam (**10**)(C=O stretch, 1758 cm^{-1}) could be prepared in good yield. Grubbs′ metathesis[15] of (**10**) yielded the achiral spirocyclic target (**11**)(C=O stretch, 1755 cm^{-1})(Scheme 1). The structure of (**11**) was confirmed by single crystal X-ray crystallography (CCDC 171676. See http://www.rsc.org/suppdata/cc/b1/b111627b/ for crystallographic files in .cif or other electronic format).

The problem with the introduction of the alkyl group at the bicylic lactam stage presumably reflects the enhanced stability of more substituted enolates.[14] The mono-alkylated thiophene lactam was thus prepared *via* a modified route in which the alkyl group was introduced earlier in the synthesis. This route proved to be straightforward and the 4-ethyl-6-methanesulfonyl fused thiophene γ-lactam (**15**) was synthesised in 6 steps from 2-nitrothiophene in 35% unoptimised overall yield (Scheme 2). The higher yield in the conversion of (**14**) to (**15**) relative to (**7**) to (**9**) may be indicative of a Thorpe–Ingold effect, but may also reflect the relative stabilities of the products.

All the fused thiophene γ-lactam targets reported here were found to cause significant inhibition of HNE and PPE. IC_{50} values of 20 μM and 150 μM were obtained for (**9**), which is not alkylated at C-4, under our standard assay conditions with HNE and PPE respectively. (**9**) was shown to be a competitive inhibitor of elastase and, as for the other thiophene-lactam



Scheme 1 Synthesis of 5,5-fused thiophene lactams. *Reagents and conditions*. I. ^{t}BuOK, THF, $ClCH_2CN$, −78 °C, 44%; II. Fe, AcOH, Ac_2O, rt, 95%; III. ^{t}BuLi, THF. 0 °C, $MeSO_2Im$, TfOMe, THF, 0 °C; IV. MeOH, NH_4OH, 85%; V. aq KOH, 2N, reflux, 58%; VI. HBTU, CH_3CN, $^i Pr_2(Et)N$, 38%; VII. ^{t}BuLi, CH_2CHCH_2I, THF, 50%;VIII. Grubb's catalyst, C_6H_6, reflux, 76%.

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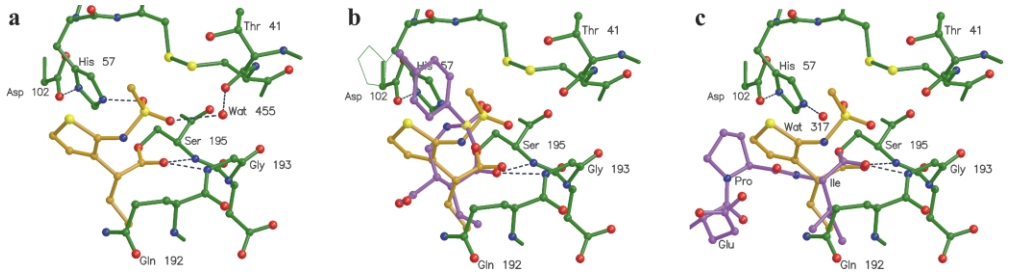
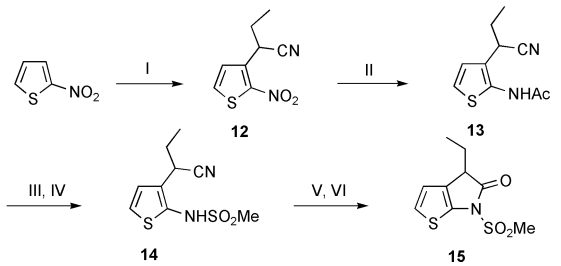


Fig. 1 Views derived from crystal structures of the active site of inhibited PPE (in green) showing (a) the 4-ethyl fused thiophene lactam (**15**) (in gold) covalently linked *via* an ester bond to Ser-195, and also overlayed with the acyl-enzyme complexes formed between (b) a monocyclic γ-lactam inhibitor[7] in purple (which displaces His-57 by *ca.* 90°, shown in thin lines) and (c) a productively bound heptapeptide, human β-casomorphin-7,[16] in purple (the hydrolytic water molecule, Wat-317, which is displaced in the inhibitor structures is shown).



Scheme 2 Synthesis of the 4-ethyl 5,5-fused thiophene lactam. *Reagents and conditions.* I. ^{t}BuOK, THF, EtClCHCN, −78 °C, 96%; II. Fe, AcOH, Ac_2O, rt, 81%; III. ^{t}BuLi, THF, 0 °C, $MeSO_2Im$, TfOMe, THF, 0 °C; IV. MeOH, NH_4OH, 50%; V. aq KOH, 2N, reflux; VI. HBTU, CH_3CN, $^{i}Pr_2(Et)N$, 89%.

inhibitors, ESI MS analyses with PPE were consistent with the formation of an acyl-enzyme inhibitor complex. The MS analyses indicated that reactivation of inhibited enzyme could occur in the case of (**9**), although whether this is due to recyclisation of the γ-lactam or hydrolysis of the acyl-enzyme is uncertain. The bis-allylated thiophene lactam (**10**) was less active than (**9**) *vs*. both PPE (IC_{50} = 120 μM) and HNE (IC_{50} > 500 μM). Slow binding kinetics were observed in the case of PPE. This together with the relatively poor inhibition observed for (**10**) is presumably due to steric hindrance of the bis-alkyl group, possibly coupled to the relatively flat nature of the bicyclic template of these inhibitors. The C-4 ethylthiophene (**15**) gave a similar IC_{50} value (20 μM) to the C-4 unalkylated compound (**9**). The most promising result was achieved with the spiro-thiophene lactam (**11**), which is a potent achiral inhibitor of HNE with an IC_{50} value of 1 μM. The conversion of the di-allyl compound (**10**) to the spirocycle (**11**) presumably provides a better fit into the S_1 binding pocket of HNE.

The ethylthiophene lactam (**15**) formed a complex with PPE that was analysed by X-ray crystallography. Native PPE crystals were soaked for 24 h in a saturated solution of the thiophene inhibitor (**15**). The soaked crystals were isomorphous to native PPE and there was no significant change in unit cell parameters. The resultant 1.4 Å high resolution structure (Fig. 1) revealed that the γ-lactam ring of the inhibitor had been opened and there was well-defined electron density consistent with an ester link between the inhibitor and Ser-195. All atoms for the thiophene inhibitor were well defined (average *B*-factor = 18.9 Å^2) with the ester carbonyl oxygen located in the oxyanion hole within hydrogen bonding distance of the amido-nitrogens of Gly-193 and Ser-195 (2.88 and 2.83 Å respectively). The C-4 ethyl group was positioned in the S_1 subsite and the thiophene ring was located approximately parallel to His-57. The C-4 chiral centre appears to be in the *R* configuration. One of the sulfonamide oxygen atoms was within hydrogen bonding distance to N_2 of His-57 (2.87 Å) and the other appeared to form a hydrogen bond with the carbonyl of Thr-41 bridged by Wat-455. No hydrolytic water molecule (as seen in the PPE:β-casomophin-7 peptide acyl-enzyme structure[16]) was observed within hydrogen bonding distance of His-57. This makes it unlikely that deacylation can occur through direct hydrolysis of the ester bond leading to release of the ring-opened thiophene molecule. Although the presumed angle between the lone pairs on the sulfonamide nitrogen atom and the ester carbonyl group makes reformation of the γ-lactam ring and deacylation in this manner possible, the potency observed for these thiophene inhibitors may, in part, be due to deactivation of the nitrogen atom by resonance stabilisation with the thiophene ring.

In summary, we have prepared a new generic template for serine protease inhibition *via* a succinct route. MS and X-ray crystallographic analyses provide evidence that the compounds form acyl-enzyme complexes. Modification of the C-4 substituent should allow selectivity for other proteases to be obtained. It is likely that the improved potency of these inhibitors *via* stabilisation of the acyl-enzyme complexes and better recognition can be achieved by functionalisation of the thiophene ring.

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