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DESIGN AND SYNTHESIS OF NOVEL MONOCYCLIC ß-LACTAM INHIBITORS OF PROSTATE SPECIFIC ANTIGEN

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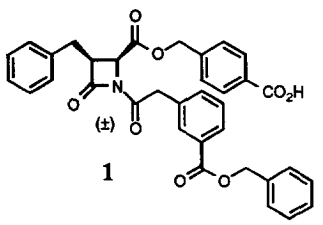
Abstract: A novel series of monocyclic ß-lactam analogues was designed using a homology derived model of prostate specific antigen (PSA) and by application of a multiple copy simultaneous search technique. Syntheses were conducted by assembly of the ß-lactam core *via* a Staüdinger reaction with elaboration at the 1, 3 and 4 positions to probe active site binding. Inhibition against PSA was evaluated.

The serine protease, prostate specific antigen (PSA), has been extensively used as a diagnostic marker for prostate carcinoma.[1, 2] Also, PSA exhibits proteolytic action on insulin-like growth factor binding protein-3 (IGFBP-3) which may contribute to malignant growth of the prostate.[3, 4] A causative rather than merely descriptive role of PSA in growth stimulation has been suggested by such proteolytic activity and also by direct stimulation of prostate epithelial cells.[5] This, and the recent discovery that PSA is implicated in breast tumours make PSA inhibitors important synthetic targets.[6]

There are no literature reports of PSA inhibitors on which to base a structural modification program. However, monocyclic ß-lactams have commonly been used to inhibit serine proteases[7] and studies in our laboratories produced **1**, which was shown to exhibit good PSA inhibitory activity (IC_{50} = 8.98 μM). This lead inhibitor was shown from peptide mapping and electrospray ionization mass spectrometry experiments to form a stable acyl enzyme complex at the active site serine 189 of PSA. Consequently, **1** was chosen as a starting point for the investigation of the binding of such monocyclic ß-lactam inhibitors to PSA, with a view to producing more potent inhibitors.



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No X-ray crystallographic data has been reported for PSA but a high degree of homology with the kallikrein family of enzymes allowed the construction of a homology derived model. The model used in this work appears similar to other homology derived models which have subsequently been described in the literature.[8-10] Molecular models of both enantiomeric structures of **1** were created within QUANTA®[11] and subjected to energy minimization and molecular dynamics calculations in CHARMm 23.1.[11] A discrete number of low energy conformations were realized for each enantiomer and a total of five structures were considered as possible realistic descriptions of inhibitor binding.

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Figure 1: MCSS results for toluene ligand on PSA active site Connolly surface

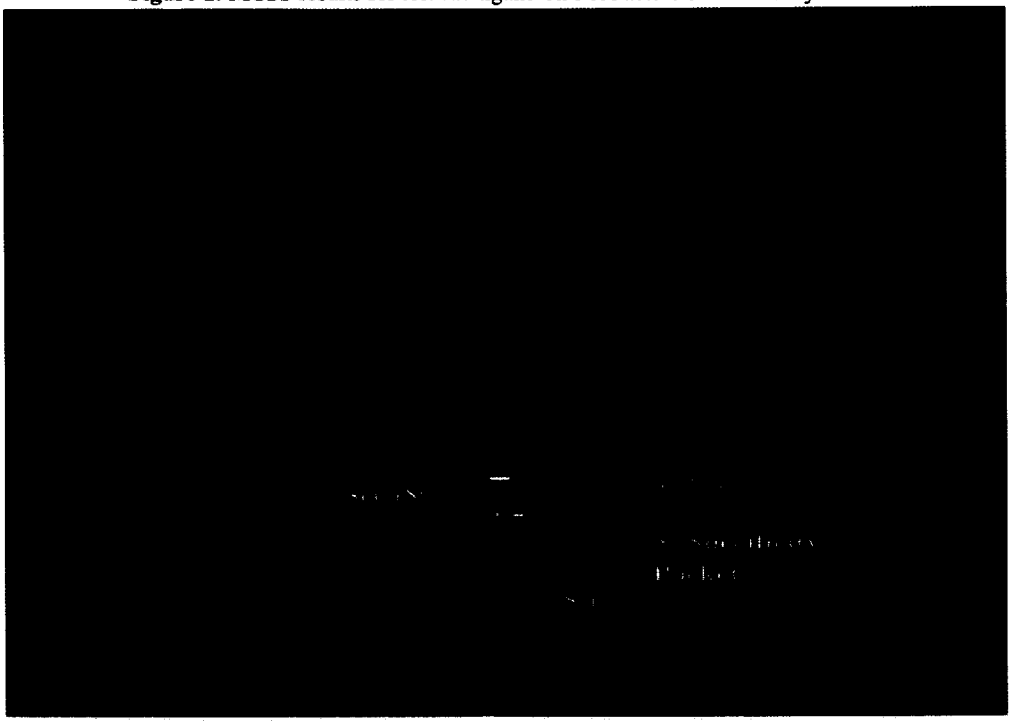
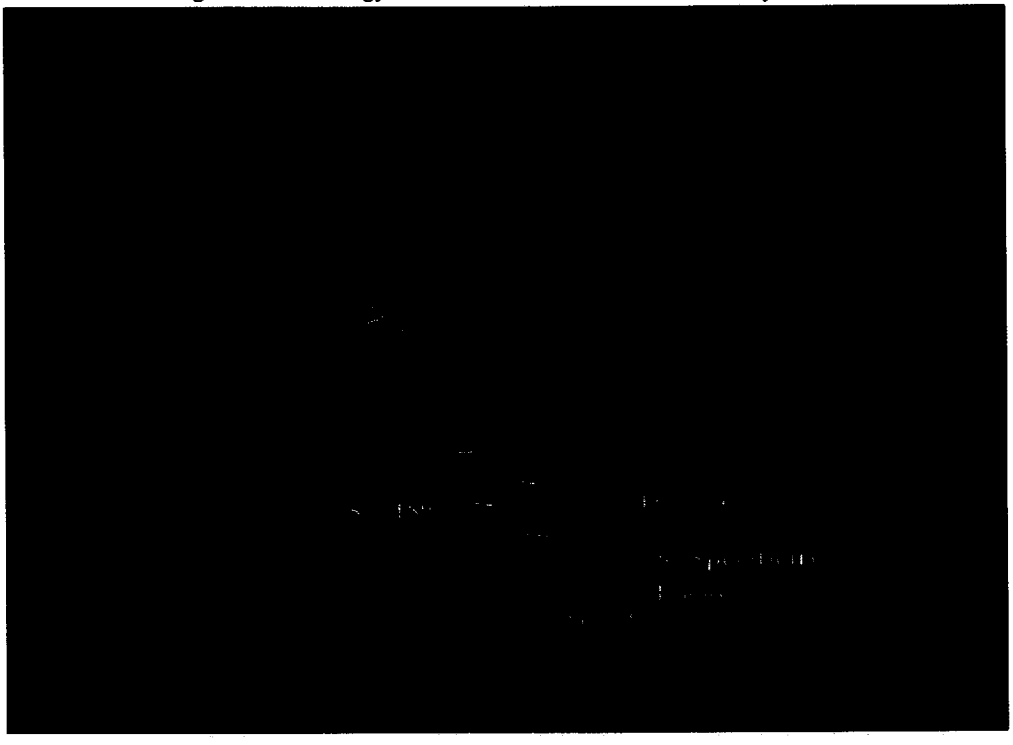


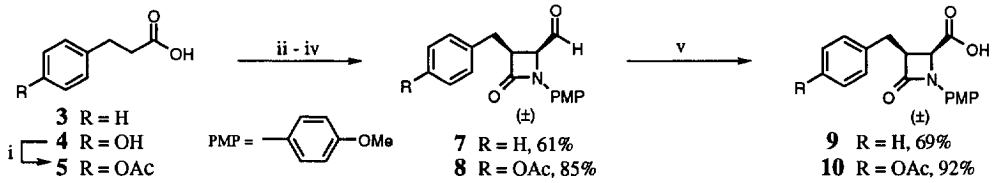
Figure 2: Low energy structure **2** on PSA active site Connolly surface



Further narrowing of binding possibilities was required before structure modification could be attempted. A multiple copy simultaneous search (MCSS) method was applied to the active site region of PSA using a toluene ligand.[12] The MCSS results identified two low energy binding subsites for the toluene ligand. The binding energy interaction increases (from green to red), as depicted in figure 1. A solvent accessible surface of the active site of PSA was calculated according to the method of Connolly.[13] Firstly, the lowest energy cluster (red and yellow) fills the S_1 specificity pocket and binding of the C-3 benzyl ß-lactam side chain of **1** in this area was common to all low energy structures under consideration. The secondary binding subsite (green ligand) shows only a single low energy form, indicating a snug fit of toluene at this position. Only one of the low energy structures, **2**, utilized this binding subsite with an excellent fit at the active site (figure 2). Thus, this structure also predicted the *(3S,4S)* enantiomer to be more active and was used for subsequent structure modification to improve inhibitor binding and PSA inhibition.

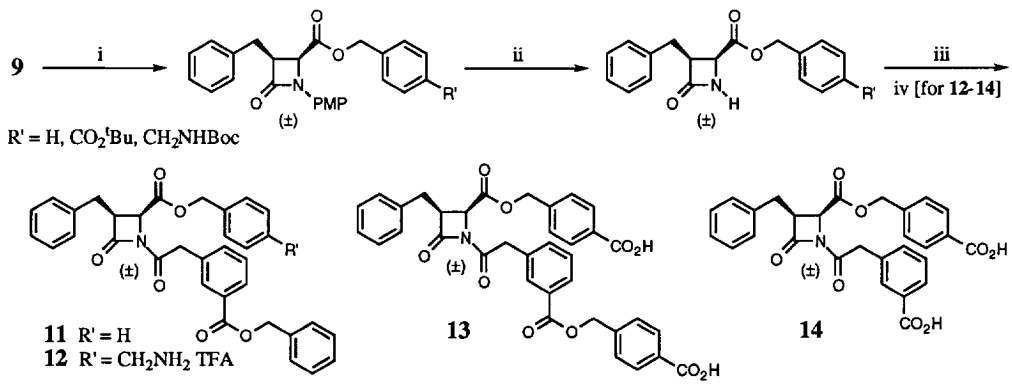
Synthesis: A monocyclic ß-lactam core with *cis* relative stereochemistry was assembled *via* a Staüdinger reaction using a modification of the method of Alcaide (scheme 1).[14] Both hydrocinnamic acid **3** and 3-(4-hydroxyphenyl)propionic acid **4** (after protection as phenolic acetate **5**) were converted into acid chlorides and treated with diimine **6**[15] in the presence of triethylamine. Subsequent oxidation of aldehydes **7** and **8** afforded acids **9** and **10** using a slight excess of Jones reagent.[16, 17]

Scheme 1: Assembly of ß-lactam core



Reagents and Conditions: i. NaOH (2.5 eq.), H_2O then Ac_2O, 0 °C, 80%; ii. R = H, PCl_5, 100°C, 80%; R = OAc, $(COCl)_2$, DCM, cat. DMF, 90%; iii. NEt_3, (*p*-MeO-C_6H_4-N=$CH)_2$ (**6**), 35-40 °C; iv. 5% HCl; v. CrO_3-H_2SO_4, acetone-H_2O, 0 °C

Scheme 2: Synthesis of C-3 benzyl ß-lactams

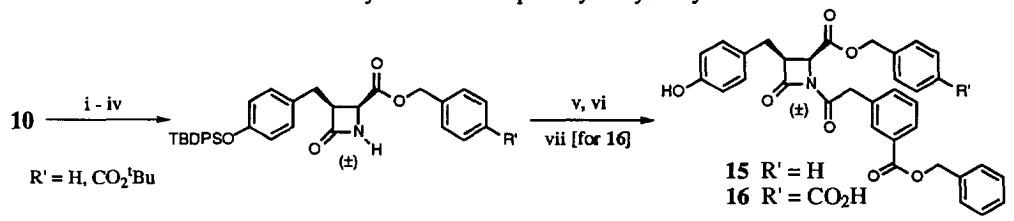


Reagents and Conditions: i. DCCI, cat. DMAP, *p*-R'-C_6H_4-CH_2OH, THF, 69-89%; ii. $Ce^{IV}(NH_4)_2(NO_3)_6$, MeCN-H_2O, 0 °C, 71-88%; iii. $NaN(SiMe_3)_2$, THF, -78 °C then *m*-R"-C_6H_4-CH_2COCl, -78 °C to RT, 36-61%; iv. CF_3CO_2H, 0 °C, 63-99%

For the C-3 benzyl ß-lactam **9**, functionalization at C-4 was achieved by DCCI mediated coupling and dearylation at nitrogen was performed using ceric ammonium nitrate (scheme 2).[18] The N-1 side chains, responsible for activation of the ß-lactam carbonyl towards serine acylation, were prepared as acid chlorides and attached to the unsubstituted ß-lactam nitrogen using sodium bis(trimethylsilyl)amide. Thus, **11** was obtained and **12**, **13** and **14** subsequently prepared by deprotection with trifluoroacetic acid.

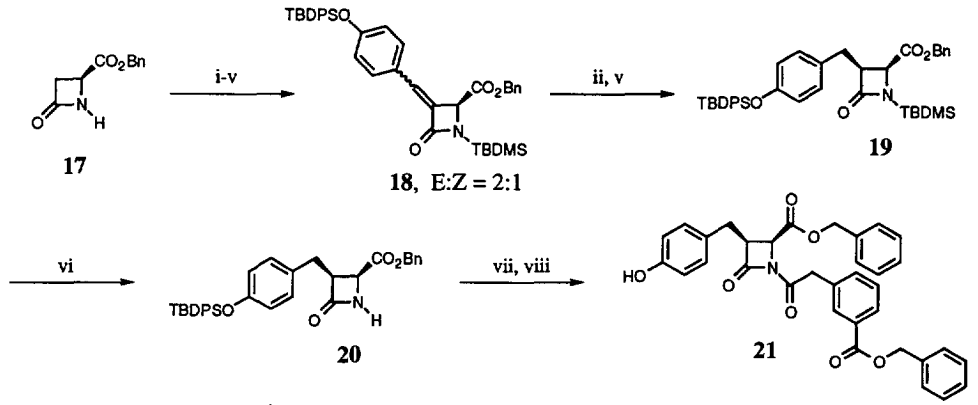
In the case of C-3 *para*-hydroxybenzyl ß-lactams, a similar synthetic sequence could be applied except that the phenolic acetate was found to require replacement by *tert*-butyldiphenylsilyl ether to allow successful nitrogen dearylation with ceric ammonium nitrate (scheme 3). Removal of the acetyl group under mild activated zinc conditions minimized competing C-4 benzyl ester hydrolysis.[19] An additional silyl ether deprotection was necessary for the formation of **15** and **16**, and was achieved without opening the ß-lactam ring using ammonium hydrogen difluoride.[20]

Scheme 3: Synthesis of C-3 para-hydroxybenzyl ß-lactams



Reagents and Conditions: i. DCCI, cat. DMAP, *p*-R'-C_6H_4-CH_2OH, THF, R' = H, 82%, R' = $CO_2{}^tBu$, 75%; ii. activated zinc, MeOH-THF, 35 °C, R' = H, 88%, R' = $CO_2{}^tBu$, 71%; iii. TBDPSCl, imidazole, DMF, 40 °C, R' = H, 94%, R' = $CO_2{}^tBu$, 78%; iv. $Ce^{IV}(NH_4)_2(NO_3)_6$, MeCN-H_2O, 0 °C, R' = H, 63%, R' = $CO_2{}^tBu$, 68%; v. $NaN(SiMe_3)_2$, THF, -78 °C then *m*-BnO_2C-C_6H_4-CH_2COCl, -78 °C to RT, R' = H, 56%, R' = $CO_2{}^tBu$, 52%; vi NH_4F•HF, NMP-DMF, R' = H, 68%, R' = $CO_2{}^tBu$, 82%; vii. CF_3CO_2H, 0 °C, 98%

Scheme 4: Synthesis of *(3S,4S)* enantiomer **21**



Reagents and Conditions: i. EtN^iPr_2, TBDMSCl, DCM; ii. H_2, Pd-C, THF; iii. LDA (2.1 eq.), THF, -9 ° C then TMSCl (1.1 eq.), THF, -9 °C, then LDA (1.1 eq.), THF, -9 °C to 0 °C; iv. *p*-TBDPSO-C_6H_4-CHO, THF, 0 °C; v. EDCI, BnOH, cat. DMAP, DCM, **17**→**18**, 20%; **18**→**19**, 32%; vi. 48% aq. HF, MeCN, 93%; vii. $NaN(SiMe_3)_2$, THF, -78 °C then *m*-BnO_2C-C_6H_4-CH_2COCl, -78 °C to RT, 45%; viii NH_4F•HF, NMP-DMF, 67%

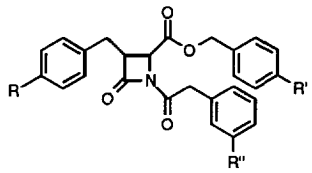
The homochiral ß-lactam **17**[21] was transformed into C-3 arylidene ß-lactam **18** using a Peterson reaction then hydrogenation and subsequent benzylation afforded **19** as the sole isolable product (scheme

4).[22] Selective desilylation with hydrofluoric acid gave **20** which was elaborated as before to give the single enantiomer **21**, $[\alpha]_D^{22.5}$ -14.1 (c 0.18 in $CHCl_3$).

Results and Discussion: The role of the C-4 side chain was first investigated in the predicted binding conformation **2** for compounds **1**, **11** and **12**. In this non covalently bound form the distance from the carboxyl group in **1** to glutamine 166 was not close enough to form a formal hydrogen bond (2.6 Å). However, on serine acylation concomitant conformational changes in both the inhibitor and protein may allow such an interaction to occur. Incorporation of an amino methyl group at this position would give more flexible directionality and a shorter distance for a similar interaction. This was revealed by the increased potency of **12**, indicating that the previous carboxyl group in **1** was not greatly contributing to binding. Indeed, in **1**, solvation of this carboxyl group out of the enzyme may disfavour binding. When no polar functionality is present at this position, as in **11**, a purely hydrophobic interaction is possible where the C-4 side chain is tightly bound in a surface groove of PSA proximal to the active site, and improved inhibition is again achieved.

The extended N-1 side chain was found essential for PSA inhibition with the second aryl ring reaching the secondary toluene binding subsite identified by MCSS calculations. In the absence of this second aryl group, **14** was inactive against PSA. A *para*-carboxyl group can be added to this second aryl ring to allow a weak polar interaction with lysine 145 providing **13**, a more potent inhibitor than **1**.

Table: Inhibition of PSA by monocyclic ß-lactams



Compound	Stereochemistry	R	R'	R''	IC_{50} (μM)[a, 23]
1	*(3S,4S), (3R,4R)*	H	CO_2H	CO_2Bn	8.98 [0.90]
11	*(3S,4S), (3R,4R)*	H	H	CO_2Bn	1.43 [0.19]
12	*(3S,4S), (3R,4R)*	H	CH_2NH_2 TFA	CO_2H	1.34 [0.05]
13	*(3S,4S), (3R,4R)*	H	CO_2H	*p*-CO_2CH_2-C_6H_4-CO_2H	5.84 [0.92]
14	*(3S,4S), (3R,4R)*	H	CO_2H	CO_2H	>30 [-]
15	*(3S,4S), (3R,4R)*	OH	H	CO_2Bn	0.348 [0.05]
16	*(3S,4S), (3R,4R)*	OH	CO_2H	CO_2Bn	3.5 [0.4]
21	*(3S,4S)*	OH	H	CO_2Bn	0.226 [0.01]

[a] IC_{50} for inhibition of 50% of the cleavage of *para*-nitroanilide from MeO-Suc-Arg-Pro-Tyr-pNA•HCl by PSA. Standard errors of the mean are quoted in parenthesis

Initially the C-3 benzyl side chain was found to be a good fit within the S_1 specificity pocket, as proposed by previous studies on ß-lactam inhibition of serine proteases, and was left unaltered.[7 (a)-(c)] However, MCSS calculations showed that it would be possible to bind this aryl ring further inside the S_1 specificity pocket. In addition, a methanol ligand binding site near the bottom of this pocket was also identified by MCSS experiments, utilizing a hydrogen bonding framework with threonine 184 and serine 217. Consequently, incorporation of a *para*-hydroxyl group at C-3 gave significantly improved PSA inhibitiors, **15** and **16,** over the respective C-3 benzyl analogues, **11** and **1**. The single enantiomer **21** showed a further improvement in PSA inhibition as predicted by the modeling rationale.

In summary, we have developed a descriptive model of the binding of a series of monocyclic ß-lactam inhibitors to PSA. The use of a MCSS technique has supplemented our model and allowed the prediction of stronger binding and more potent PSA inhibitors. A full description of our active site analysis of PSA and complete synthetic details for the preparation of a full range of monocyclic ß-lactam inhibitors will be published in due course.

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23. 10 µl of each inhibitor was pre-incubated with 10 µl of PSA (40 nM), in 10 µl of phosphate buffered saline/bovine serum albumen (BSA) (6.3µg/ml BSA), for two hours at 37 °C. 70 µl of 1mM substrate was added and serial dilution to give half-maximal effect of cleavage evaluated.

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