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THROMBIN INHIBITORS BASED ON [5,5] *TRANS*-FUSED INDANE LACTAMS

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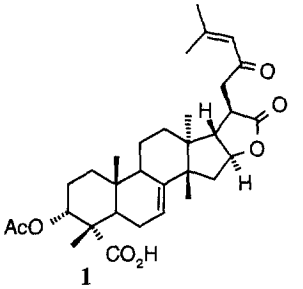
Abstract. A series of *trans*-fused lactams containing the indane nucleus has been prepared. Compound **19** has much enhanced plasma stability compared with its lactone counterpart and shows appreciable *in vitro* anticoagulant activity.

An inhibitor of the pro-coagulant serine protease thrombin would have potential benefit in a variety of thrombotic disorders.[1,2]

We have previously described our efforts to find a selective, orally active compound based on the [5,5] trans-fused lactone template as found in the natural product **1** which was isolated from extracts of *Lantana Camara* and which inhibits thrombin with an IC_{50} of 4 nM.[3] This class of compounds inhibits thrombin by acylating the active site serine 195[4] *via* the strained lactone ring.



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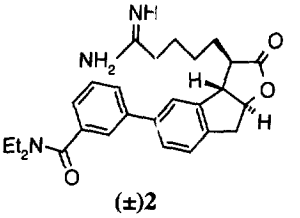

(±)2

A number of simplified analogues of **1**, containing the strained *trans*-lactone function, were prepared.[5] Subsequently the indane lactones, e.g. **2**, were synthesised, examples of which were found to be potent acylating inhibitors of thrombin *in vitro* (compound **2** has an apparent second order rate constant for enzyme inactivation of 11 x 10^6 $M^{-1}sec^{-1}$).[6] Compound **2** bears an amidine containing side-chain which was found to enhance potency by forming an ionic interaction with the acidic aspartate 189 of thrombin. However, the limited stability of the lactone function to hydrolysis in plasma indicated that these compounds would have little utility as therapeutic agents. In an effort to improve the stability of the [5,5] *trans*-fused system we decided to prepare the lactam analogues. The general synthesis of indane lactams is outlined in Scheme 1.[7]

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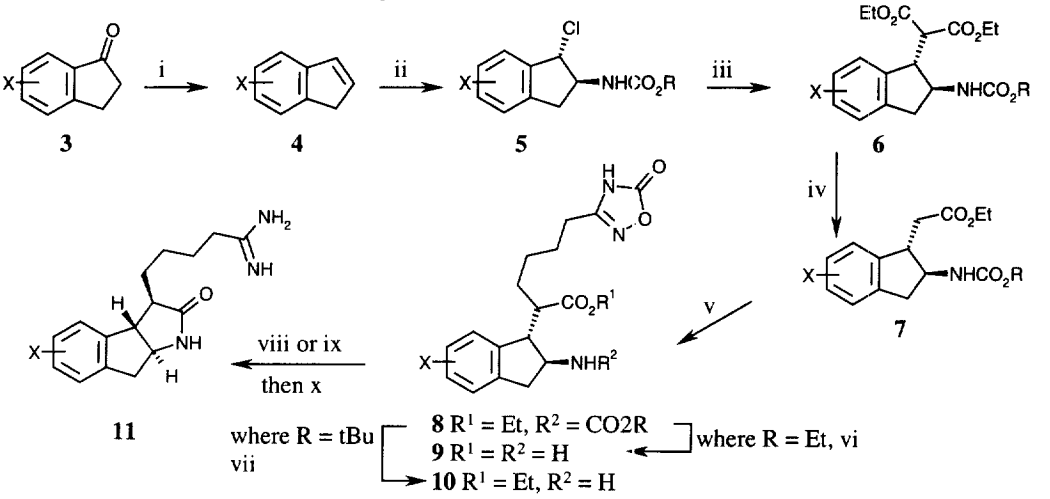
Scheme 1: Synthesis of trans-fused indane lactams



Conditions: (i) a. $NaBH_4$, EtOH, 59-89% b. p-toluenesulfonic acid, toluene, 80°C, 29-77% (ii) Cl_2NCO_2R, toluene then aqueous $NaHSO_3$ 74-82% (iii) NaH, $CH_2(CO_2Et)_2$, DMF, 40°C, 96-97% (iv) NaCl, H_2O, dimethylsulfoxide, 160°C, 55-85% (v) a. lithium bis(trimethylsilyl)amide, THF, -78°C b. alkyl iodide[5], THF, HMPA, -78°C to room temp., 37-81% (vi) $Ba(OH)_2$, EtOH, H_2O, reflux, 69-96% (vii) trifluoroacetic acid, CH_2Cl_2, 90-95% (viii) 1-methyl-2-chloropyridinium chloride, Et_3N, CH_2Cl_2, reflux, 31-60% (ix) diphenylphosphoryl azide, Et_3N, DMF, 63% (x) H_2, Pd-C, EtOAc, AcOH, 62-96%

The appropriately substituted 1-indanone **3** was converted to the indene **4** which was treated with either ethyl or *tert*-butyl N,N-dichlorourethane[8] to give, after reductive work-up, the *trans*-1-chloro-2-aminoindane derivative **5** (R = Et or *t*Bu, respectively). Addition of diethyl malonate anion to this system provided the *trans*-ester **6**, presumably *via* an aziridine intermediate, and decarboxylation then gave the ester **7**. Where X = Br a variety of substituted phenyl rings could be introduced onto the benzenoid ring by means of a palladium catalysed boronic acid coupling performed on this ester **7** under non-aqueous conditions.[9] The amidine containing side chain could then be introduced in its protected form[10] by α-alkylation with the previously described alkyl iodide.[5] This procedure resulted in a mixture of diastereoisomers as determined by ^1H NMR spectroscopy. Lactamisation was subsequently performed using either 1-methyl-2-chloro-pyridinium chloride[11] or diphenylphosphoryl azide followed by hydrogenation to unmask the amidine function. The desired and predominant β diastereoisomer was obtained pure by preparative HPLC.

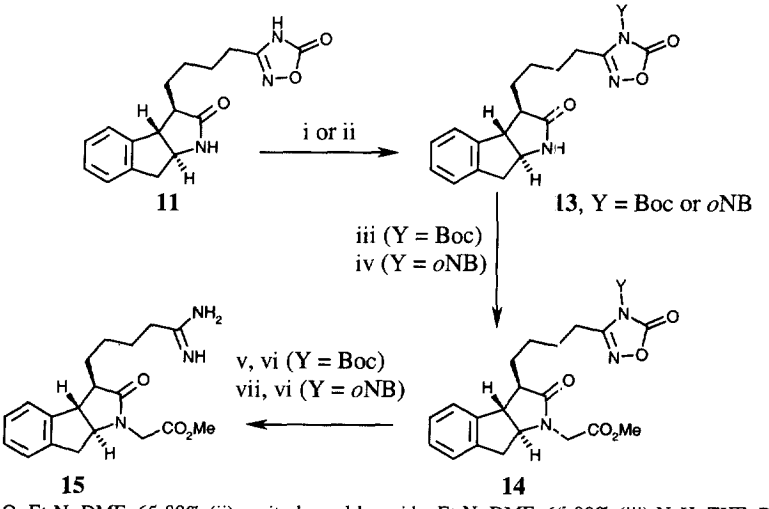
The unsubstituted indane lactam **11** (X = H) although displaying good stability ($t_{1/2}$ >24h) in human plasma was found to be inactive as a thrombin inhibitor (IC_{50} >200μM).[12] The inhibitory activity of acylating inhibitors is dependant on two components: the initial recognition event in which a non-covalent complex (Michaelis complex) is formed between the inhibitor and the enzyme active site and the subsequent acylation of the serine 195 residue.[13] We reasoned that the loss of activity observed with lactam **11** was due to the much reduced reactivity of the lactam function relative to the lactone function. We believed that the loss of reactivity could be compensated for in two ways:

- By enhancing the binding affinity of the template to thrombin by accessing additional binding sites
- By increasing the reactivity of the lactam carbonyl group towards nucleophilic attack by serine 195

The S′ binding sites of thrombin are believed to be involved in the interaction of thrombin with one of its natural substates[14] and have been characterised by the binding of a series of peptidic transition state inhibitors.[15] Briefly S1′ is a restricted pocket accepting only small amino acid residues. However, in close proximity to the S′ is the amino acid residue lysine 60f which, amongst the trypsin serine protease family, is unique to thrombin. The S2′ pocket is a lipophilic region capable of accommodating a phenyl ring. We therefore attempted to substitute on the lactam nitrogen to probe for an interaction with the lysine 60f residue or with the lipohilic S2′ pocket.

The N-phenylpentyl substituted lactam **12** was prepared by reductive amination of intermediate amine **10** with the appropriate aldehyde ($Ph(CH_2)_4CHO$, $NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$, 80-90%) followed by hydrolysis of the ester function and subsequent steps as described in Scheme 1. To enable the introduction of the CH_2CO_2Me substituent, it was first necessary to protect the acidic nitrogen of the oxadiazolinone group with either the *tert*-butoxycarbonyl (Boc) or *ortho*-nitrobenzyl group (*o*NB) (Scheme 2). The lactam nitrogen could then be alkylated and the protecting groups removed to give compound **15**.

Scheme 2: N-Alkylation of indane lactams



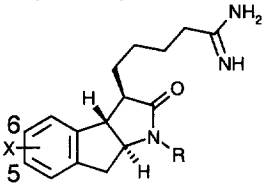
Conditions: (i) $(Boc)_2O$, Et_3N, DMF, 65-80% (ii) *o*-nitrobenzyl bromide, Et_3N, DMF, 65-80% (iii) NaH, THF, $BrCH_2CO_2Me$, 37% (iv) NaH, DMF, $BrCH_2CO_2Me$, 62% (v) trifluoroacetic acid, CH_2Cl_2, 100% (vi) H_2, Pd-C, EtOAc, AcOH, 60-80% (vii) UV, dioxan, 100%

Both N-substituents were found to increase potency significantly (Table 1, compounds **12** and **15**) but it is noteworthy that the ester substituent conferred significant thrombin selectivity as might have been expected if an interaction with lysine 60f had indeed been achieved.

The introduction of the (3-diethylaminocarbonyl)phenyl substituent onto the 5- or 6- position[16] of the benzene ring *via* a palladium mediated boronic acid coupling of appropriate bromoindane **7** (X = Br) followed by

introduction of the N-alkyl group by reductive alkylation as described above and further steps as outlined in Scheme 1 gave compounds **16** and **17**. Incorporation of the substituted phenyl group resulted in a slight increase in potency (see Table 1) which was disappointing in light of the pronounced increase in potency seen for related analogues in the lactone series.[6]

Table1. Enzyme inhibitory activity of *trans*-fused [5,5] indane lactams



	X	R	IC_{50} v Thrombin (μM)[12]	IC_{50} v Trypsin (μM)[12]	IC_{50} v FXa (μM)[12]
11	H	H	>200	>200	>200
12	H	$(CH_2)_5Ph$	13	9.7	>100
15	H	CH_2CO_2Me	43	>200	>200
16	6-(3-Et_2NOC)Ph	$(CH_2)_5Ph$	5	>50	>50
17	5-(3-Et_2NOC)Ph	$(CH_2)_5Ph$	5	8.4	26
18	6-(3-Et_2NOC)Ph	CO_2Me	0.05	2.2	2.2
19	5-(3-Et_2NOC)Ph	CO_2Me	0.016	0.03	0.12
20	6-(3-Et_2NOC)Ph	$CO_2(CH_2)_3Ph$	0.13	2.3	3.7
21	5-(3-Et_2NOC)Ph	$CO_2(CH_2)_3Ph$	0.66	0.62	3.6
22	6-(3-Et_2NOC)Ph	CONHEt	3.0	>100	>100
23	6-(3-Et_2NOC)Ph	$CONMe_2$	11	>20	>20
24	6-(3-Et_2NOC)Ph	COEt	0.38	8.3	9.9
25	6-(3-Et_2NOC)Ph	CH_2OAc	12	36	14
26	6-(3-Et_2NOC)Ph	SO_2Ph	0.016	0.066	0.074
27	5-(3-Et_2NOC)Ph	CONHEt	5.6	1.7	5.6
28	5-(3-Et_2NOC)Ph	$PO(OEt)_2$	3.2	1.7	20

Our second approach to increase potency was to enhance the reactivity of the lactam function towards attack by the serine 195 hydroxyl, by substituting on the lactam nitrogen with electron withdrawing groups. Thus the N-alkoxycarbonyl substituents in compounds **18**, **19**, **20** and **21** were incorporated by an analogous method to that outlined in Scheme 2 using the appropriate alkoxycarbonyl chloride. It was gratifying to see that the two methoxycarbonyl substituted analogues **18** and **19** both exhibited substantially improved potency (Table 1). The more lipophilic compounds **20** and **21** were attempts to both activate the lactam carbonyl to attack by serine 195 and to access the lipophilic S2′ pocket. However, these latter two compounds exhibited weaker activity than **18** and **19**. This suggests that the terminal phenyl ring does not make an optimal contribution to binding in these carbamate substituents.

We were able to determine the structure of the complex between compound **18** bound covalently to thrombin by X-ray crystallography[17] (Figure 1). Some general features are worthy of comment. As expected the lactam group acylated the active site serine 195 and the amidine side chain occupied the S1 pocket and made an ionic

interaction with the asparate 189 residue. The diethylcarboxamide substituent on the pendant phenyl ring was seen to occupy the lipophilic S3 pocket formed by tyrosine 215, leucine 99 and isoleucine 174. It is noteworthy that in the acylated enzyme complex the indane core was almost orthogonal to that found in complexes of the corresponding lactones[6] and thus it made no interaction with S2 pocket defined by histidine 57, tyrosine 60a and tryptophan 60d. To compensate there was a clear hydrogen-bonding interaction between the carboxamide carbonyl oxygen and the hydroxyl of tyrosine 60a, an interaction not possible with the other serine proteases which do not possess this residue. It is interesting to speculate that this latter interaction may be the source of the increased selectivity of **18** over **19** but unfortunately we were unable to obtain good quality X-ray data for the latter compound.

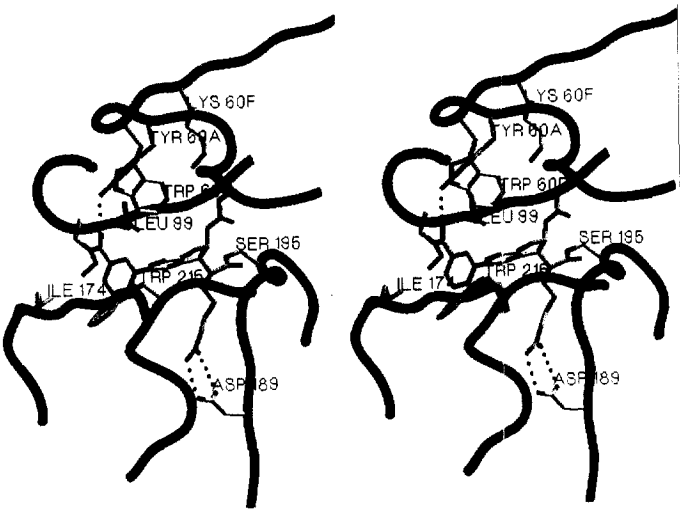


Figure 1: A stereo view of the active site showing the protein backbone (green) with some key residues interacting with compound 18. Key hydrogen bonds are indicated by dotted lines.

The potency of **18** and **19** prompted us to carry out a full kinetic analysis on these compounds. The observed second order rate constants for inhibition of thrombin by these two compounds were found to be 3.0 x 10^4 and 6.5 x 10^4 M^{-1} sec^{-1} respectively compared to 1100 x10^4 and 42 x 10^4 M^{-1} sec^{-1} respectively for the two corresponding lactone analogues.[6] The *in vitro* anticoagulant activities of **18** and **19** were determined by the standard APTT protocol. Whereas compound **18** was a poor anticoagulant, doubling the APTT at 200 μM, the 5-aryl substituted lactam **19** showed appreciable activity, doubling the APTT at 15 μM. This may be due to a combination of greater inhibitory potency v. thrombin and the related procoagulant activated factor X, and the increased stability of **19** in human plasma ($t_{1/2}$ 24h c.f. 17h for **18**). Unfortunately, compound **19** was still considered to be insufficiently potent in the APTT assay to be progressed further.

In order to fine tune the reactivity of the lactam a range of other N-substituents was prepared, by analogous methods to Scheme 2, using an appropriate electrophile, and the results are included in Table 1. The only substituent that apparently combined the appropriate electronic and spatial characteristics for inhibition was the

phenylsulfonyl group in compound **26**. This compound showed good potency against thrombin but which also suffered from insufficient anticoagulant activity, doubling the APTT at 35μM.

Conclusion

Whilst these compounds possess insufficient anticoagulant activity to warrant further progression, the information they have provided regarding the factors governing potency and selectivity have enabled us to build on the findings in a structurally distinct series. These studies will be reported in due course.

Acknowledgements

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12. IC_{50} is the concentration of compound required to inhibit 50% of the enzyme-catalysed hydrolysis of the chromogenic substrate; Tos.Gly.Pro.Lys.-pNA for thrombin and S222 for trypsin and factor Xa. The compounds were incubated with the enzyme for 15 min prior to the addition of the substrate. Reported values are the mean of triplicate determinations.
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16. For convenience we have retained the indane numbering system throughout.
17. Crystals of thrombin/hirugen were soaked overnight with excess compound **18**. X-ray data were collected to 2.5Å and the structure refined to an R-factor of 19%.