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SYNTHETIC [5,5] TRANS-FUSED INDANE LACTONES AS INHIBITORS OF THROMBIN

Martin Pass*, Richard E. Bolton, Steven J. Coote, Harry Finch, Sean Hindley, Andrew Lowdon, Edward McDonald, Jessica McLaren, Martin Owen, Neil A. Pegg, Christopher J. Mooney, Chi-Man Tang, Simon Parry and Champa Patel

Glaxo Wellcome Research and Development, Gunnels Wood Rd., Stevenage, Herts SG1 2NY, United Kingdom

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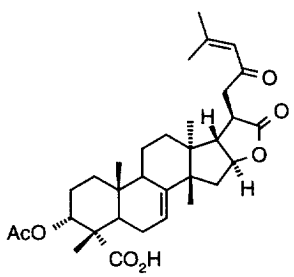
Abstract. Synthesis of *trans*-fused lactones containing the indane nucleus has resulted in a series of potent acylating inhibitors of thrombin. As an example compound **11e** has an apparent second order rate constant of 11×10^6 $M^{-1}sec^{-1}$ for the inhibition of thrombin. The anticoagulant activity of these compounds is discussed.

Introduction

The search for selective, orally active inhibitors of the pro-coagulant serine protease thrombin has been the focus of much effort in recent years since such an inhibitor would have potential benefits for the treatment of a variety of thrombotic disorders.[1, 2]

Our strategy for the discovery of a non-peptide lead molecule employed high throughput screening of natural products and compounds from our sample collection. A series of euphane triterpenes exemplified by **1**, isolated from extracts of *Lantana Camara* was identified as an active lead.[3] Compounds of this class inhibit thrombin by acylation of the active site serine 195 *via* the strained lactone ring.[3] Simplification of this structure led us to the racemic [5,5] *trans*-lactone **2** which inhibits thrombin with an IC_{50} of 0.13 μM.[4] In an effort to improve on the potency of **2** we wanted to reintroduce more of the planar hydrophobic nature of the original triterpene leads. This Letter describes the synthesis and activity of a series of indanes developed from **2** some of which are very potent inactivators of thrombin *in vitro*.



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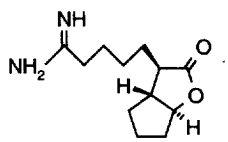

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* E-mail: mp8073@glaxowellcome.co.uk; fax: +44 (0)1438 763616

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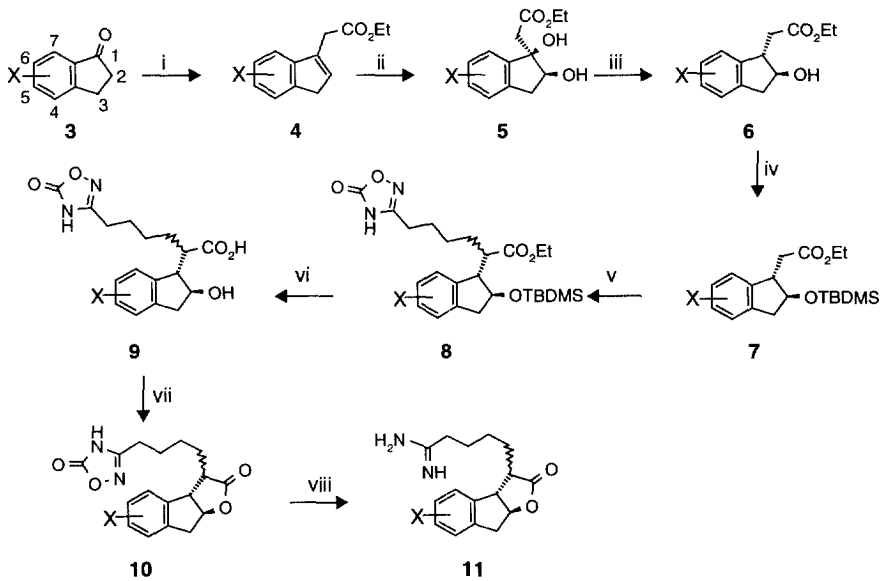
Chemistry

Compounds **11a-j** were prepared according to the general route outlined in Scheme 1.[5] Thus the appropriately substituted indan-1-one **3** was converted *via* a multistep sequence into the hydroxy acid **9** which contains a protected amidine function.[6] A variety of substituted phenyl groups could be incorporated as substituents to the benzenoid ring by means of a palladium catalysed boronic acid coupling performed on **7**, **9** or **10**, where X = Br. This reaction was generally carried out using tetrakis(triphenylphosphine)palladium (0) in aqueous dimethoxyethane at 85°C.[7] However when the substrate contained the sensitive lactone function the coupling was carried out using non-aqueous conditions ($Pd(OAc)_2$, $(o\text{-}tol)_3P$, Et_3N, DMF, 65°C).[8] The amidine function was released from its protected form by hydrogenolysis or zinc-acetic acid mediated reduction.

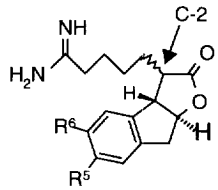
Scheme 1



Conditions: (i) a. NaH, $(EtO)_2POCH_2CO_2Et$, THF, 40-72% b. lithium diisopropylamide (LDA), THF then phosphate buffer, 50-100% (ii) OsO_4, N-methylmorpholine-N-oxide, acetone, tBuOH, H_2O, 21-85% (iii) $BF_3.Et_2O$, Et_3SiH, CH_2Cl_2, 36-61% (iv) *tert*-butyldimethylsilylchloride (TBDMSCl), imidazole, DMF, 55-97% (v) LDA then alkyl iodide[4], HMPA, THF, 44-67% (vi) KOH or NaOH, EtOH 66-96% (vii) 2,4,6-trichlorobenzoyl chloride, Et_3N, CH_2Cl_2[9] then 4-(dimethylamino)pyridine, $PhCH_3$, 10-33% (viii) H_2, Pd/C, AcOH, EtOAc or Zn, AcOH, 22-80%.

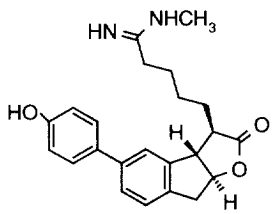
The N-methyl analogue **12** was prepared *via* methylation (NaH, MeI, DMF) of the appropriate oxadiazolinone **10**. The chain-shortened analogue **13** was prepared according to Scheme 1 using the appropriate alkyl iodide. Finally the α-methyl substituted compound **14** was obtained as a 35:65 β/α mixture of diastereoisomers by α-methylation of ester **7** (LDA, MeI, HMPA, THF) followed by the usual transformations.

Table 1. Substituted *trans*-fused [5,5] indane lactones



11	R^5	R^6	Stereochemistry at C-2 (β/α)[a]
a	H	H	94:6
b	H	Ph	92:8
c	H	(4-HO)Ph	>98:2[b]
d	H	(3-Ph)Ph	93:7
e	H	(3-Et_2NCO)Ph	>98:2[b]
f	Ph	H	9:1
g	(3-MeO)Ph	H	>4:1
h	(3-Ph)Ph	H	93:7
i	(3-Et_2NCO)Ph	H	98:2
j	(4-HO)Ph	H	>98:2[b]

a. ratio determined by ^{1}H NMR, b. no α-isomer was detected



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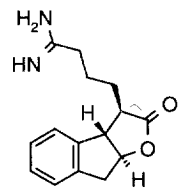
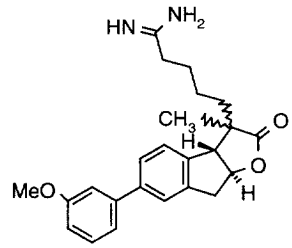


13



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Results and Discussion

In order to obtain a meaningful structure-activity relationship for the inhibitory activity of these compounds against thrombin and the related serine protease trypsin it was necessary to perform a full kinetic analysis. Thus all compounds with IC_{50} <1 μM in our initial chromogenic assay[10] were fully evaluated by progress curve analysis.[3] The results are presented in Table 2 in the form of the apparent second order rate constant (k_{obs}/[I]) for enzyme inactivation.

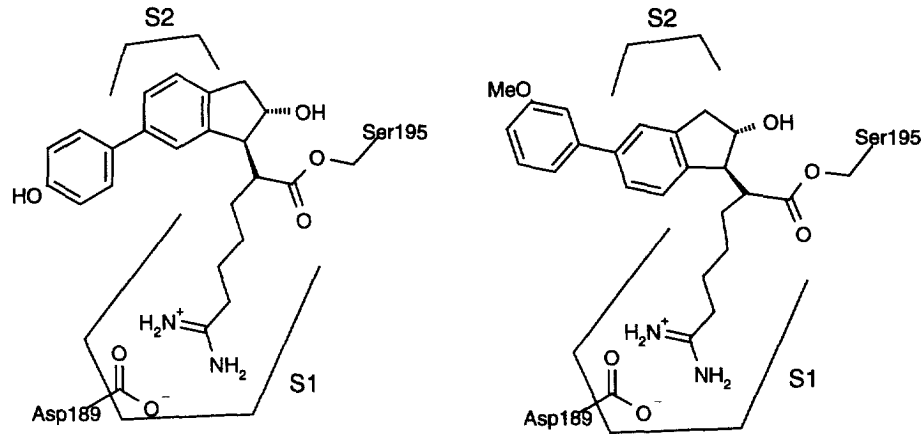
Table 2. Enzyme inhibitory and anticoagulant activity of *trans*-fused [5,5] indane lactones

	k_{obs}/[I] v. thrombin ($x10^6$ M^{-1} s^{-1})[a]	k_{obs}/[I] v. trypsin ($x10^6$ M^{-1} s^{-1})[a]	APTT (2xcontrol) (μM)	Selectivity (Thr/Tryp)
PPACK[b]	16[c]	NT	1.8[d]	
11a	0.0042	NT	100	
11b	0.49	NT	60	
11c	0.27	NT	42	
11d	3.8	0.11	85	35
11e	11	0.20	40	55
11f	0.048	0.020	50	2.4
11g	0.096	0.036	25	2.7
11h	0.16	NT	NE	
11i	0.42	0.22	4	1.9
11j	0.033	NT	27	
12	IC_{50} 1.6 μM	NT	NE	
13	IC_{50} 0.6 μM	NT	NT	
14	IC_{50} 1.8 μM	NT	NE	

NT = not tested, NE = no significant effect at 100μM, a. see ref 11, b. see ref 12, c. see ref. 13, d. see ref 1

It is apparent that substitution is well tolerated at both the 5 and 6-position[14] with some examples exhibiting inhibitory activity comparable with the standard thrombin inhibitor PPACK.[12, 13] However, substitution at the 6-position generally leads to more potent inhibitors of thrombin *in vitro*, together with significant selectivity for thrombin over the closely related serine protease trypsin. Inspection of the covalent complexes of compounds **11c** and **11g** with human thrombin, determined by X-ray crystallography, may provide an explanation for this trend.[15] We have found that in these acyl-enzyme complexes the amidine group binds *via* a salt-bridge with aspartate 189[16] at the bottom of the S1 pocket. Compound **11c** has its pendant phenyl ring directed towards the hydrophobic S3 pocket formed by leucine 99, isoleucine 174, and tryptophan 215 (Figure 1). This conformation allows the central aromatic portion to partially occupy the hydrophobic S2 pocket formed by tyrosine 60a, tryptophan 60d, and histidine 57. The 5-substituted compound **11g**, whilst binding in a grossly similar manner, does not occupy the S2 pocket, at least in the acyl-enzyme complex, probably due to the pendant aryl ring preventing any movement further into the S2 pocket. If these features were present in the early non-covalent complex (Michaelis complex) and/or the transition state for the acylation step then they would lead to faster inactivation of the enzyme by 6-substituted compounds by stabilising the early recognition complex or the transition state for acylation. X-ray crystallography of the complex of **11i** with thrombin reveals a hydrogen-bond between the carboxamide carbonyl oxygen in compound **11i** with the hydroxyl of tyrosine 60a.[15] This may explain the enhanced potency of this compound relative to the rest of the 5-substituted series. It is interesting to speculate that a similar interaction may be responsible for the activity of **11e** although we were unable to obtain good X-ray data for this compound.

Figure 1: The binding of compounds of compounds 11c and 11g to the active site of thrombin



Changes to the amidine-containing side chain failed to produce compounds with enhanced potency. Thus N-methylation of the amidine resulted in a very weakly active compound **12**, a direct analogue of **11c** (IC_{50} 9nM). Likewise shortening the amidine-bearing side-chain of the unsubstituted compound **11a** (IC_{50} 77nM) resulted in compound **13**, which was also a very weak inhibitor. Both of these changes are expected to interfere with the crucial amidine-carboxylate coulombic interaction with the aspartate residue 189 at the bottom of the S1 specificity pocket. Retrospective modelling studies suggested that methylation at the α-position of the lactone ring as in compound **14**, an analogue of compound **11g** (IC_{50} 18nM), is likely to be detrimental due to a steric clash in the active site. In light of these findings we were discouraged from preparing the corresponding analogues with the optimum 6-substituent as in **11e**.

We tested our compounds for their ability to retard the coagulation of human plasma. The concentration of the test compound necessary to double the activated partial thromboplastin time (APTT) was used as our standard measure and these results are included in Table 2. Generally the APTT results for the 6-substituted series were disappointing despite their $k_{obs}/[I]$ values, especially when compared with their 5-substituted counterparts. For example, **11b** and **11i** had very similar $k_{obs}/[I]$ values but **11b** was more than ten-fold less active in the APTT assay. Interestingly, improved anticoagulant activity was seen for both **11b** and **11i** when the preincubation time was reduced in the APTT test protocol (data not shown). We ascribe these findings to the increased instability of 6-substituted compounds in plasma relative to the 5-substituted series. Two direct analogues, **11e** and **11i**, had half-lives in human plasma of less than 1 minute and 120 minutes respectively. We assume that a hydrolytic plasma enzyme (unidentified) mediates the hydrolysis of the strained lactone ring and that it exhibits some specificity for the 6-substituted compound. We attempted to increase stability by α-substitution since it

was hoped that the added steric hindrance would reduce susceptibility to enzymatic hydrolysis. Unfortunately, as already reported, compound **14**, although more stable ($t_{1/2}$ 402 min), was only a weak thrombin inhibitor.

Conclusions

A series of [5,5] *trans*-fused lactones based on the indane template has been synthesised. Although some of these compounds exhibit excellent potency *in vitro* their instability in plasma preclude them from further evaluation. Building on our X-ray structural data we have put in place a strategy designed to overcome this instability and our results will be described in due course.

Acknowledgements

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5. All of the new compounds are racemic and exhibit satisfactory spectra (^{1}H NMR, IR) and mass spectral or combustion analytical data.
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10. 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. The compounds were incubated with the enzyme for 15 min prior to the addition of the substrate.
11. The apparent second order rate constant, $K_{obs}/[I]$, is calculated as the mean of at least three determinations. Standard deviations were less than 10% of the mean.
12. PPACK is D-Phe-Pro-Arg-chloromethylketone
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14. For convenience we have retained the indane numbering system throughout.
15. Jhoti, H.; Cleasby, A.; Reid, S.; Thomas, P. J.; Weir, M.; Wonacott, A. manuscript in preparation.
16. Trypsin amino acid numbering is used.