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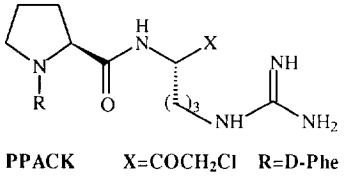
DESIGN AND SYNTHESIS OF CONFORMATIONALLY CONSTRAINED ARGINAL THROMBIN INHIBITORS [1]

Aldo Salimbeni,[a*] Fabio Paleari,[a] Renato Canevotti,[a] Marco Criscuoli,[b] Annalisa Lippi,[b] Mauro Angiolini,[c] Laura Belvisi,[c] Carlo Scolastico,[c] Lino Colombo[d]

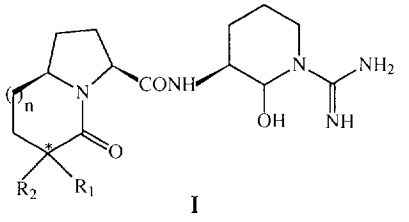
[a]Lusofarmaco,[†] Medicinal Chemistry Department, 20132 Milano, [b]Laboratori Guidotti,[†] Pharmacology Department, 56010 Pisa, [c]University of Milano, Organic and Industrial Chemistry Department, C.N.R. Centre for the Study of Organic and Natural Compounds, 20133 Milano, [d]University of Pavia, Pharmaceutical Chemistry Department, 27100 Pavia, Italy

Abstract: A series of conformationally constrained arginal thrombin inhibitors was prepared starting from 5,6 or 5,7 bicyclic lactamic structures, that an indirect approach of X-ray structure-based drug design indicated as D-Phe-Pro dipeptide mimetics. The tetrahydroquinolyl sulfonamido derivative **Ig** (LR-D/009) displayed the best inhibitory potency (IC_{50} = 0.018 μm), with good selectivity over plasmin and trypsin. © 1997 Elsevier Science Ltd.

Thrombin is a trypsin-like serine protease, produced during activation of blood coagulation pathways, which plays a key role in the coagulation process.[2] It regulates hemostasis, stimulates platelet aggregation and catalyses the conversion of fibrinogen into fibrin. Pathological conditions resulting in inefficient control of thrombin activity may induce the formation of intravascular thrombotic disorders. Consequently inhibition of thrombin by either an indirect or direct mechanism of action has become a major target in the development of therapies for these conditions.[3] Starting from the prototypical irreversible thrombin inhibitor D-Phe-Pro-Arg chloromethylketone[4] (PPACK, Figure1), several related arginal tripeptides, based on the same aa sequence, have been synthesized and have been shown to be effective and reversible active site directed thrombin inhibitors.[5]



PPACK X=$COCH_2Cl$ R=D-Phe
BMY 44621 X=CHO R=desNH_2-Phe
GYKI 14766 X=CHO R=CH_3NH-D-Phe



I

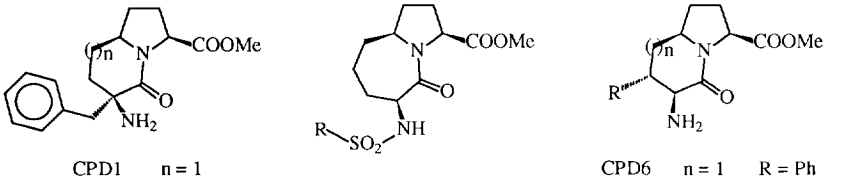
Figure 1. Structures of tripeptide thrombin inhibitors and general formula of the new compounds.

In a recent paper Semple et al.[6] reported a series of structurally related thrombin inhibitors in which a fused thiazolidine lactam was successfully incorporated into the peptide backbone. This result prompted us to publish our work in this area. According to a molecular modelling study based on the X-ray crystallographic data of complexes of thrombin with known inhibitors, we found that the bicyclic lactamic structures shown in Figure 2

[†] Company related to A. Menarini, Industrie Farmaceutiche Riunite.

Fax: ++39-39-5300008

could act as conformationally restricted Phe-Pro dipeptide mimetics. With the aim of increasing the conformational rigidity in the arginal tripeptide inhibitors, while reducing their peptidic properties, we synthesized a series of compounds of general formula **I** (Figure 1 and Table 3), based on some of the bicyclic peptidomimetic templates reported.



CPD1 n = 1
CPD2 n = 0
CPD3 n = 2

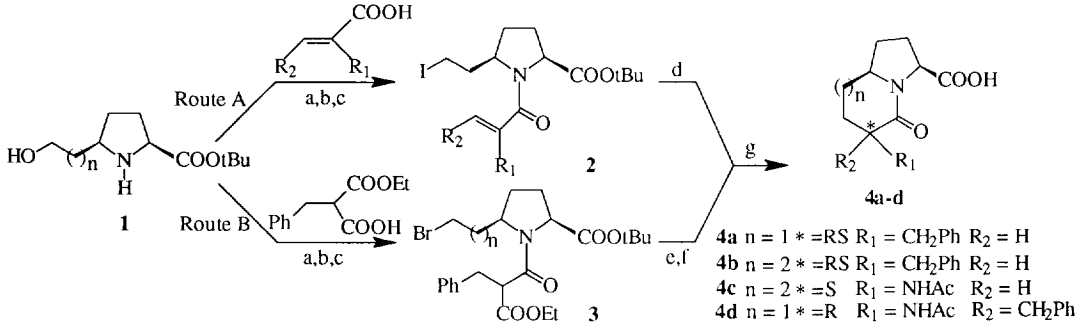
CPD4 R = 1- Naphthyl
CPD5 R = CH_2Ph

CPD6 n = 1 R = Ph
CPD7 n = 2 R = CH_2Ph
CPD8 n = 2 R = Ph

Figure 2. New proposed bicyclic structures.

Chemistry

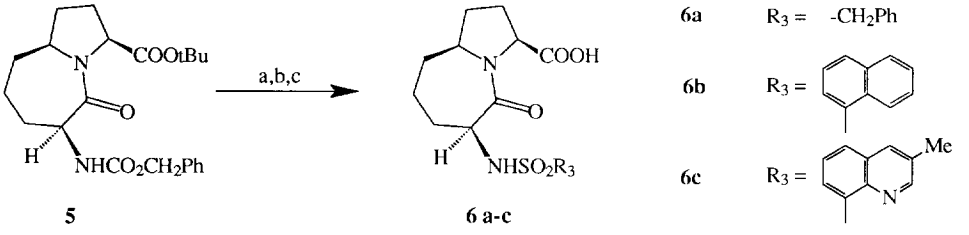
The 5,6- and 5,7-bicyclic lactams **4a-d** and **6a-c**, the key intermediates for the synthesis of thrombin inhibitors **Ia-g**, were prepared starting from the known pyrrolidines **1**[7] following routes A or B, depending on the nature of the substituents R_1 and R_2 (Scheme 1).



Scheme 1. (a) DCC/THF/DMAP, 50÷65%; (b) $MsCl/Et_3N/CH_2Cl_2$, 97%; (c) NaBr or NaI/acetone/Δ, 89%; (d) $Bu_3SnH/AIBN/C_6H_6/\Delta$, 33÷54%; (e) NaH/THF, 82%; (f) NaOH (aq)/MeOH, 85%; (g) CF_3COOH, 92%.

The first approach (Route A) was based on the regio- and stereoselective radical cyclization of 2-iodoethyl-N-alkenoylpyrrolidines **2** and led to 5,6- and 5,7-bicyclic lactams **4c,d** carrying an amino protected group. The stereochemical outcome of the cyclization was investigated by NMR studies (N.O.E. experiments) and it was found to be strictly dependent on the nature of R_2.[8] Only the 7-*endo-trig* bicyclic isomer **4c** was produced in good yield when R_2 was hydrogen. On the contrary, phenyl substituted N-alkenoyl pyrrolidine **2** (R_2 = Ph) gave, exclusively, the 6-*exo-trig* isomer **4d** in moderate yield. Bicyclic lactams **4a,b** possessing a benzylic appendage were conveniently prepared by non stereoselective ionic cyclization starting from bromoalkyl-N-acylpyrrolidines **3** (Route B). The bicyclic sulfonamido derivatives **6a-c** were synthesized in good yields following the three-step

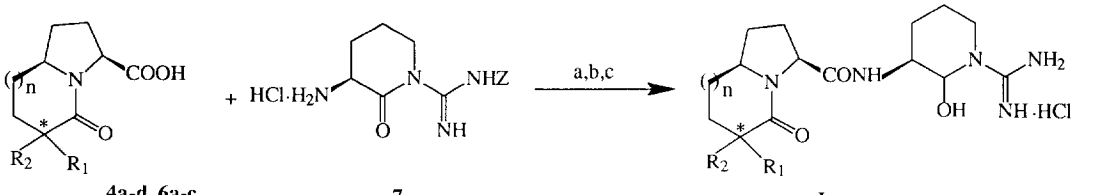
procedure in Scheme 2, starting from the octahydropyrrolo[1,2-a]azepinone t-butylester **5** obtained from **2** (R_1 = $NHCOOCH_2Ph$; R_2 = H) according to Scheme 1 (Route A).



5 **6 a-c**

Scheme 2. (a) H_2/Pd-C 10%/MeOH, 92%; (b) $R_3SO_2Cl/Et_3N/CH_2Cl_2$, 89%; (c) CF_3COOH, 93%.

Finally, target thrombin inhibitors **Ia-g** were obtained according to a modified reported procedure via mixed-anhydride coupling of **4a-d** and **6a-c** with the arginine derivative **7**[9] followed by lactam reduction with $LiAlH_4$ at -20°C and hydrogenolysis of the guanidine protective group (Scheme 3). ^{1}H-NMR studies have revealed that the arginals **Ia-g** exist in solution mainly in the cyclic emiaminal form.



4a-d, 6a-c **7** **I a-g**

Scheme 3. (a) iBuOCOCl/NMM/DMF/-20°C, 72%; (b) $LiAlH_4$/THF/-20°C, 61%; (c) H_2/Pd-C 10%/THF/HCl (aq), 75%.

Molecular Modelling

As part of a project directed towards the discovery of novel, nonpeptidic active-site inhibitors of thrombin, we used an indirect approach of X-ray structure-based drug design to suggest conformationally restricted mimetics of the D-Phe-Pro dipeptide fragment, that has been shown to be of great importance in thrombin active site recognition.[10] The structure of the irreversible inhibitor PPACK bound to the human α-thrombin in the X-ray complex, solved by Bode et al.[4] at 1.9 Å resolution (PDB code 1PPB), was selected as the reference for our modelling study, which was undertaken within the framework of the MacroModel/BatchMin V4.5[11] program.

Our strategy to increase conformational rigidity of the peptide backbone was based on the incorporation of properly functionalized bicyclic lactam scaffolds as mimetics of the P2 and P3 lipophilic portions of PPACK or its reversible analogs. The proposed 5,5-, 5,6- and 5,7-fused bicyclic lactamic structures (Figure 2) derive from the idea of tethering the D-Phe side chain C-β atom to the Pro ring C-δ atom, while exploring different solutions for both the tether length and the position of the aromatic group designed to interact with the hydrophobic S3 thrombin pocket. The mimetic properties of the novel conformationally constrained bicyclic lactams were first evaluated on the basis of 7-point rigid fits of MM2* conformers[12] to the X-ray structure of PPACK bound to the

thrombin active site, according to the criteria shown in Figure 3. The results, expressed as root mean square (RMS) deviation of the 7 superimposed atoms, are reported in Table 1, where lower RMS values indicate a better superimposition.

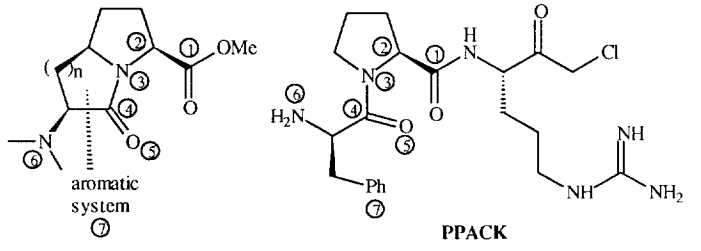


Figure 3. Scheme of the 7-point superimposition PPACK-bicyclic derivatives.

Table 1. 7-point rigid fit results.

Compound	RMS (Å)
CPD1	0.199
CPD2	0.211
CPD3	0.130
CPD4	0.668
CPD5	0.614
CPD6	0.407
CPD7	0.829
CPD8	0.380

To better rationalize the experimental results (see Table 3), additional molecular superimpositions to the lead inhibitor PPACK were performed employing different overlay criteria suggested by the X-ray crystal structures of human α-thrombin complexed with novel inhibitors incorporating P3-P4 monocyclic lactam sulfonamido moieties.[13,14] MM2* conformers of the bicyclic structures CPD1, CPD4 and CPD5 were superimposed with a 3-point rigid fit to the X-ray structure of PPACK. Points 6 and 7 of the previous scheme (Figure 3) were maintained in the new fit, while the third correspondence involved the centroid of the bicyclic scaffold and the centroid of PPACK Pro ring. The RMS values are reported in Table 2.

Table 2. 3-point rigid fit results.

Compound	RMS (Å)
CPD1	0.508
CPD4	0.364
CPD5	0.367

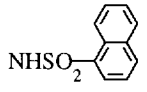
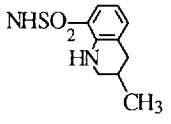
Results and Discussion

Compounds **Ia-g** and the known inhibitors BMY 44621,[9] GYKI 14766[15] and Argatroban[16] were evaluated for their ability to inhibit the activity of human α-thrombin following the method described in the literature.[17] For some of them the selectivity against the enzymes trypsin and plasmin was also determined.[17] The results are reported in Table 3.

The seven-membered lactam derivatives **Ie-g** bearing an aromatic or a heteroaromatic sulfonamido appendage on the bicyclic scaffold displayed higher inhibitory activity than compounds **Ia-d**, suggesting that P2-P4 peptidomimetic fragments such as CPD4 and CPD5 (Figure 2) ensure a better spatial functionality arrangement than dipeptide surrogates CPD1-CPD3 to interact with the S2 and S3 thrombin binding sites. Only by hypothesizing that the whole P2-P3 bicyclic lactam scaffold occupies the S2 pocket instead of PPACK Pro residue, we obtained rigid fit results (Table 2) supporting these conclusions.

According to X-ray structural data and models derived for a series of structurally related thrombin-bound inhibitors,[6,13,14] the novel P1-arginal derivatives **Ie-g** featuring conformationally constrained P2-P4 bicyclic lactam sulfonamido moieties should bind in the thrombin active site in a normal substrate-like mode and undergo all the key binding interactions with the S1, S2 and S3 pockets. The new inhibitors should form high affinity antiparallel β-sheet hydrogen bonds to Gly216 residue with the lactam carbonyl and the sulfonamide N-H. The P1-argininal side chain should make a close electrostatic contact in S1 with Asp189, and the P4-benzyl group should lie in the hydrophobic S3 pocket. The biological data of novel structurally related P2-proline, P3-monocyclic lactam and P2-P3 bicyclic lactam derivatives suggest that the interaction with the thrombin 60 loop may play a critical role in determining inhibitory activity and selectivity. A direct computational study of the interactions between inhibitors and enzyme is in progress to confirm our binding hypotheses.

Table 3. *In vitro* activities of thrombin inhibitors **I**.

Compd.[a]	R_1	R_2	n	*	IC_{50} (μM) thrombin	IC_{50} (μM) trypsin	IC_{50} (μM) plasmin
Ia	CH_2Ph	H	1	RS	4.60	ND	ND
Ib	CH_2Ph	H	2	RS	1.40	ND	ND
Ic	$NHCOCH_3$	H	2	S	4.30	24	74
Id	$NHCOCH_3$	CH_2Ph	1	R	3.90	ND	ND
Ie	$NHSO_2CH_2Ph$	H	2	S	0.11	ND	10
If		H	2	S	0.11	ND	10
Ig		H	2	S	0.018	0.1	1.04
BMY 44621[9]					1.60	7.2	41
GYKI 14766[15]					0.026	0.02	0.5
Argatroban[16]					0.039	5.0	800

[a] All the compounds were characterized by NMR, mass (FAB^+) and C,H,N elemental analyses.

The tetrahydroquinolyl derivative **Ig** was the most active in the series (IC_{50}=0.018μM), being more potent than the reference inhibitors. It also exhibited good selectivity for thrombin over plasmin, while the selectivity to trypsin was moderate but comparable to that of the other inhibitors (GYKI 14766, BMY 44621). In a preliminary *in vivo* evaluation, in a model of venous stasis thrombosis in anesthetized rat,[18] **Ig** was able to inhibit thrombus growth and significantly increase thrombin time.[19]

In conclusion we have demonstrated that a series of bicyclic lactamic structures could act as Phe-Pro dipeptide mimetics in arginal tripeptide inhibitors, in accordance with the results of other authors. In particular, the incorporation of the new tetrahydroquinolylsulfonylamino-octahydro-pyrrolo[1,2-a]azepinone carboxylic acid derivative into the peptide backbone, gave the potent and selective thrombin inhibitor **Ig** (LR-D/009), which was selected for further pharmacological evaluation.

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