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Discovery of AZD8165 – a Clinical Candidate from a Novel Series of Neutral Thrombin Inhibitors

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K. Abrahamsson,[a] P. Andersson,[a] J. Bergman,[a] U. Bredberg,[a] J. Brånalt,[a] A.-C. Egnell,[a] U. Eriksson,[a] D. Gustafsson,[a] K.-J. Hoffman,[a] S. Nielsen[a], I. Nilsson,[a] S. Pehrsson[a], M. O. Polla,†[a] T. Skjaeret,[a] M. Strimfors,[a] C. Wern,[a] M. Ölwegård-Halvarsson,[a] Y. Örtengren,[a]

A novel series of neutral thrombin inhibitors has been developed using a selection process based on docking experiments and property calculations and predictions. This work resulted in the identification of the highly potent thrombin inhibitor compound **85** (K_i = 300 pM) and the corresponding propionate prodrug **5** (AZD8165) as a candidate for clinical evaluation in the treatment of thrombosis and related diseases. AZD8165 was found to be safe and well tolerated and delivered the expected pharmacological response in a phase I trial in healthy male volunteers.

Introduction

Oral anticoagulants are an important group of medicines for treatment of thrombotic disorders like deep vein thrombosis, pulmonary embolism, prevention of stroke in patients with atrial fibrillation and secondary prevention of myocardial infarction. The vitamin K antagonist warfarin which reduces the efficiency of coagulation enzymes was for a long time the only oral anticoagulant on the market.[1] However, in the new millennium first oral direct thrombin and later oral direct FXa inhibitors have been introduced on the market. Ximelagatran was the first thrombin inhibitor approved but it was later withdrawn from market due to liver side effects. Dabigatran etexilate is now the only thrombin inhibitor on the market. More lately three oral FXa inhibitors have been approved, rivaroxaban, apixaban and edoxaban. In general the efficacy and safety of these agents appear similar.[2] Both ximelagatran and dabigatran etexilate are prodrugs based on a basic benzamidine and a carboxylic acid (figure 1). The strongly basic P1 functionality leads to modest bioavailability (20 and 3-7 % for ximelagatran and dabigatran etexilate, respectively).[3,4] Strategies to increase bioavailability by reducing basicity resulting in for example the 2-(amidinoaminooxy)ethyl and benzamidrazone moieties has been unsuccessful and permeability and bioavailability are still low for compounds containing these very polar moieties.[5,6] Attempts to increase bioavailability by increasing lipophilicity has resulted in issues

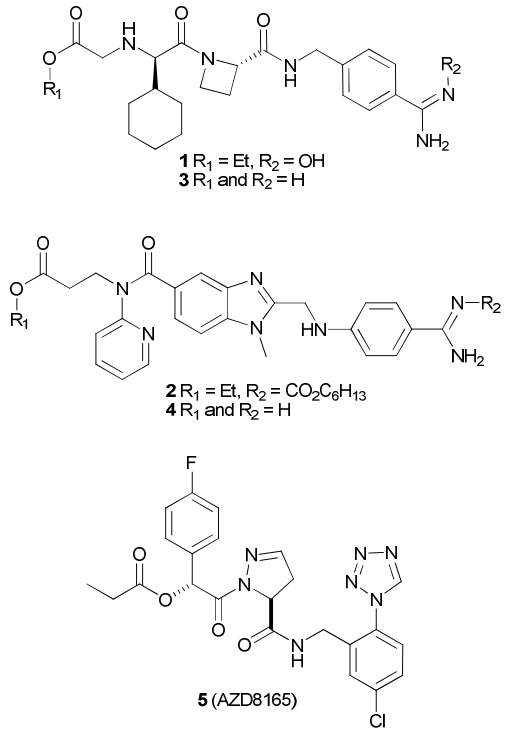

Fig. 1 Prodrugs **1** (ximelagatran) and **2** (dabigatran etexilate) and corresponding active thrombin inhibitors **3** (melagatran) and **4** (dabigatran) and **5** (AZD8165)

like low solubility, high plasma protein binding and extensive metabolism.[7,8,9,10,11] Furthermore, the hydrophilic active agents (melagatran and dabigatran) have predominantly renal excretion.[1,2] The low absorption and high renal clearance both contribute to inter-patient variability in plasma exposure and thereby risk of bleeding in the typical thrombosis patient due to age related variations in renal function. The oral direct FXa inhibitors are non-charged non-prodrugs but rivaroxaban and

[a] *Cardiovascular and Metabolic Disorders iMed, AstraZeneca R&D Mölndal, SE-431 83 Mölndal, Sweden.*
† Corresponding author
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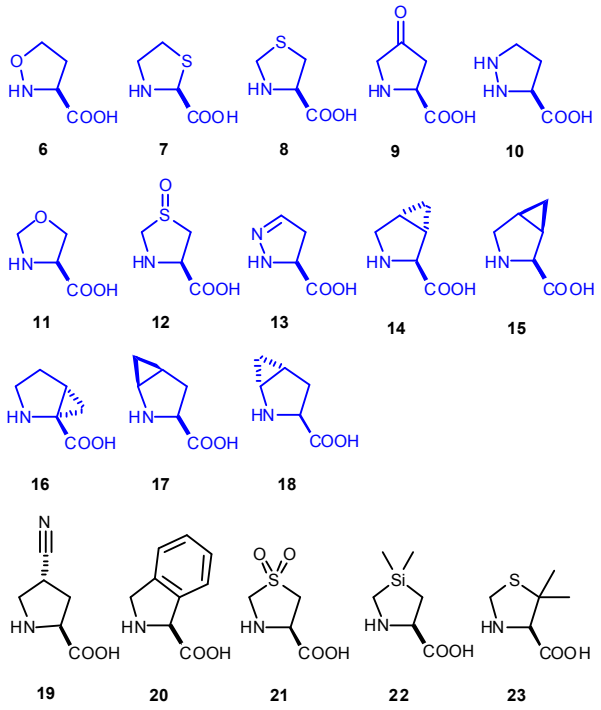

Fig. 2 Selected proline analogs. Building block **6-18**, marked blue, passed the docking criteria and was included in DMTA cycle 1.

apixaban are sensitive to CYP_{3A4} and/or P-gp inhibiting drugs which is another source of variability due to drug-drug interactions.[12,13] Neither melagatran nor dabigatran are selective against trypsin (selectivity window against trypsin I for melagatran is 2x and for dabigatran 11x) indicating that high selectivity towards trypsin may not be achieved using the benzamidine moiety.[14,15] According to the FDA label GI-side effects like dyspepsia and gastritis-like symptoms are more common compared to warfarin. The experience was the same with ximelagatran (AstraZeneca, data on file). A possible explanation is that inhibition of trypsin I, II or V prevents PAR2 activation which decreases the mucin production in the gastric wall.[16,17] Finally, thrombin inhibitors with a positive net charge tend to have acute toxicity when given intravenously, especially if lipophilic. Thus, there are several reasons to avoid benzamidine and other basic P1 substituents in development of improved oral direct thrombin inhibitors.

With this background we set out to develop a second generation oral direct thrombin inhibitor with the goals of having a neutral P1 substituent, good oral bioavailability with possibility for once daily administration (tentatively as a slow release formulation), several elimination pathways and high acute intravenous tolerability. As a result compound **5** (AZD8165)[18,19,20] was discovered, fulfilling these criteria. The selection process was based on docking experiments and property calculations and predictions in a novel series of neutral thrombin inhibitors.

Results and discussion

Design strategy

Our strategy was to base the inhibitors on the traditional D-Phe-Pro-Arg motif avoiding the planar pyrazinone- and pyridinone-type structures reported in the literature with the hope that a non-planar proline-like P2 fragment would decrease the potential problem with low solubility.[6,7,8,21] We selected a set of 18 P2 proline analogs being either commercially available or having a published synthetic route (figure 2). Furthermore, our plan was to exchange the arginine for a neutral P1 fragment. A literature survey of possible neutral P1 moieties was undertaken identifying a number of reported SAR studies. The 2, 5-dichlorophenyl group has been described to produce compounds inhibiting thrombin in the low nM range but is also very lipophilic.[22] The 2-(O-alkylacetamido)-5-chlorphenyl group was shown to result in compounds with similar potency as the 2, 5-dichlorophenyl group and is less lipophilic.[10] Finally, the (5-chloro-2-(1H-tetrazol-1-yl)phenyl group was identified from a number of aryl heterocycle P1 groups as an optimal neutral P1 moiety with a combination of low lipophilicity and subnanomolar potencies in several series of thrombin inhibitors.[‡,23] Analysis of in-house thrombin co-crystal structures suggested that a α-hydroxy-acid was an optimal neutral P3 moiety with the hydroxyl groups forming a shorter hydrogen bond to Gly-216 than was observed with P3 sulfonamide or alkylamine containing inhibitors (figure 3). We envisioned that a first design cycle would include docking studies of the set of selected P2 proline analogs combined with the P1 tetrazole and a small set of P3 α-hydroxy-acids. Three P3 α-hydroxy-acids, with phenyl, t-Bu and neopentyl side chains, where chosen for this first design cycle. Docking experiments should be able to filter out the worst behaved P2 fragments and give us a set of potentially good binding P2 fragments. After synthesis and testing of the selected compounds, the most promising P1-P2 fragments would then be combined, in a second design cycle, with a larger set of P3 α-hydroxy-acids. In this design step docking studies would be combined with property predictions and predictive models to arrive at a final set of compounds for synthesis.

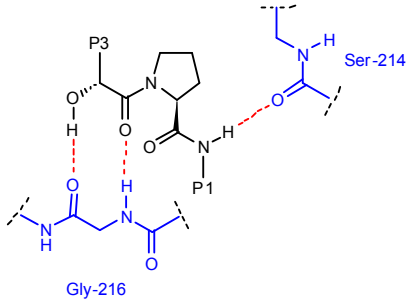

Fig. 3 Schematic inhibitor and H-bond interactions with Ser-214 and Gly-216 in the thrombin active site.

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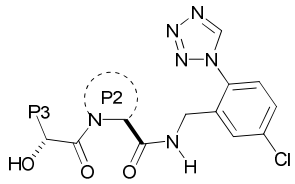
Table 1 P2 exploration

Compound **24-54**



Compound	P2	P3	Docking result[a]	Thrombin IC_{50} (nM, human)[b]	APTT IC_{50} (μM, human)[c]	Caco-2 P_{app} (10^{-6} cm/s)[d]	HLM CL_{int} (μL/min/mg)[e]
24	**11**	tBu	Pass	290		8.6	21
25	**6**	tBu	Pass	32	1.6		<12
26	**6**	phenyl	Pass	26	1.5		<12
27	**6**	neopentyl	Pass	40	1.1	25	<12
28	**9**	tBu	Pass	190		0.8	<12
29	**7**	tBu	Pass	7600		5.2	
30	**8**	tBu	Pass	26	1.2	19	120
31	**10**	tBu	Pass	27	1.7	7.2	15
32	**10**	phenyl	Pass	16	0.93	5.3	<16
33	**10**	neopentyl	Pass	24	1.3	15	25
34	**13**	tBu	Pass	28	1.4	15	<12
35	**13**	phenyl	Pass	8.0	0.84	7	<12
36	**13**	neopentyl	Pass	6.4	0.59	24	22
37	**12**	tBu	Pass	3900		0.15	<12
38	**18**	tBu	Pass	11	1.1	34	54
39	**18**	phenyl	Pass	2.5	0.68	17	<15
40	**18**	neopentyl	Pass	1.8	0.52	24	71
41	**17**	tBu	Pass	14	1.5	16	75
42	**17**	phenyl	Pass	3	0.62	8	<16
43	**17**	neopentyl	Pass	3.5	0.57	25	40
44	**14**	tBu	Pass	4.6	0.57	17	<15
45	**14**	phenyl	Pass	9.0	0.96	12	61
46	**14**	neopentyl	Pass	5.3	0.91	25	20
47	**15**	tBu	Pass	10	1.2	3.8	22
48	**15**	phenyl	Pass	2.6	0.70	2.2	<15
49	**16**	tBu	Pass	43	1.5		
50	**16**	phenyl	Pass	25	1.1	2.6	43
51	**16**	neopentyl	Pass	12	0.62	7.8	27
52	**20**	tBu	Fail	3200		9	
53	**21**	tBu	Fail	500		0.7	<12
54	**23**	tBu	Fail	1500		6.2	360

[a] Docking studies in the thrombin active site using GLIDE. [b] Inhibition of human thrombin *in vitro*. [c] Activated partial thromboplastin time. [d] Apparent permeability at pH 6.5.[24] [e] Metabolic stability in human liver microsomes.[25]

Design-Make-Test-Analysis (DMTA) cycle 1 - P2 exploration

To this end a virtual compound library of 54 compounds was first created from the set of selected building blocks.

The compounds were then docked in the active site of thrombin using GLIDE.[26] From the analysis of previous in-house crystal structures it was evident that most potent thrombin inhibitors engage in three hydrogen bonds with thrombin backbone residues. The P1-P2 amide NH is forming a hydrogen bond to the carbonyl oxygen of Ser-214 and the P3 carbonyl oxygen and hydroxyl are involved in hydrogen bonds with Gly-216 (figure 3). These three hydrogen bonds where used as a filter in the docking studies on the virtual compounds. Optimally, all three hydrogen bonds should be present in a docked low energy conformation of a successful compound.

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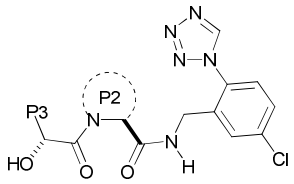
Table 2 P3 exploration

Compound **55-88**



Compound	P2	P3	Thrombin IC_{50} (nM, human)[a]	APTT IC_{50} (μM, human)[b]	Caco-2 P_{app} (10^{-6} cm/s)[c]	HLM CL_{int} (μL/min/mg)[d]
55	**13**	Benzyl	20	1.4	11	<12
56	**14**	Benzyl	18	1.2		96
57	**15**	Benzyl	8.4	0.97		74
58	**13**	Butyl	10	0.61	9.8	<12
59	**15**	Butyl	2.8	0.75	33	32
60	**18**	Butyl	6.0	0.87	35	89
61	**13**	Cyclohexyl	6.3	0.97	19	63
62	**14**	Cyclohexyl	2.0	0.85	25	110
63	**17**	Cyclohexyl	4.0	0.6	16	150
64	**14**	Cyclopropylmethyl	33	1.4	3.4	<14
65	**18**	Cyclopropylmethyl	56	0.85	6.9	25
66	**17**	Cyclopropylmethyl	52	1.7	3.6	<12
67	**13**	t-Butyloxymethyl	22	0.75		
68	**14**	t-Butyloxymethyl	5.3	0.57	17	37
69	**17**	t-Butyloxymethyl	4.4	0.5	4.5	21
70	**13**	Methoxydimethyl	44	3.7	1.7	<12
71	**15**	Methoxydimethyl	11	1.0	3.5	23
72	**17**	Methoxydimethyl	16	1.0	16	40
73	**13**	2-Fluorophenyl	32	1.1	8.9	16
74	**15**	2-Fluorophenyl	3.8	0.58	2.3	<12
75	**17**	2-Fluorophenyl	5.5	0.77	16	<12
76	**18**	3-Fluorophenyl	5.8	1.1		32
77	**13**	Methylcyclopropylmethyl	21	0.73	9.5	<12
78	**15**	Methylcyclopropylmethyl	9.6	0.50	2.2	12
79	**18**	Methylcyclopropylmethyl	9.9	1.3		
80	**15**	2-pyridylmethyl	30	1.7	0.62	
81	**17**	2-pyridylmethyl	16	1	1.2	17
82	**14**	1H-Pyrazol-1-ylmethyl	72	1.8	0.31	
83	**17**	1H-Pyrazol-1-ylmethyl	110		0.45	<12
84	**13**	3-Methylbutyl	5.8	0.63	29	47
85	**13**	4-Fluorophenyl	3	0.71	5.8	<12
86	**13**	3-Methylphenyl	4.1	0.83	9.7	<12
87	**13**	3-Cyanophenyl	25			
88	**17**	3-Chlorophenyl	1.9	1		>500

[a] Inhibition of human thrombin *in vitro*. [b] Activated partial thromboplastin time. [c] Apparent permeability at pH 6.5.[24] [d] Metabolic stability in human liver microsomes.[25]

Furthermore, all docking poses with severe clashes between inhibitor and enzyme where discarded. Thirteen of the eighteen P2 proline analogs survived these criteria and were consequently selected for synthesis (compound **6-18** in figure 2). Three additional P2 fragments, compounds **20**, **21** and **23**), with suboptimal behavior in the docking studies, where chosen as negative controls of the docking/filtration procedure and where also selected for synthesis (table 1). The docking filters were used only as a crude filter to prioritize chemistry and no attempts were made to rank compounds using scoring

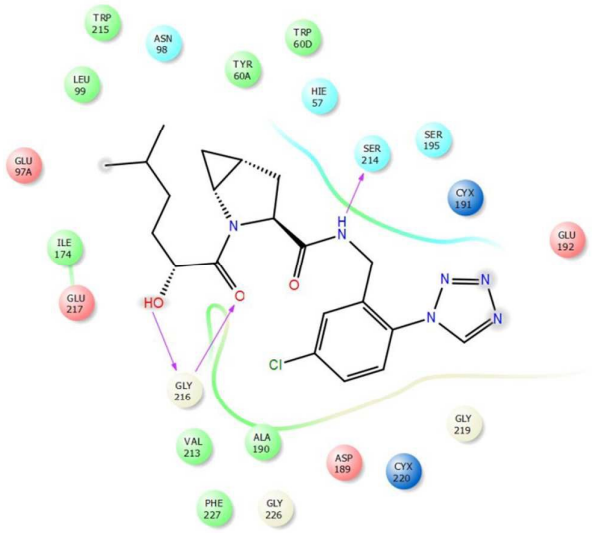


Fig. 4 2D diagram of binding pose of an example of a compound passing the criteria in the docking studies. Hydrogen bond interactions are shown in magenta.

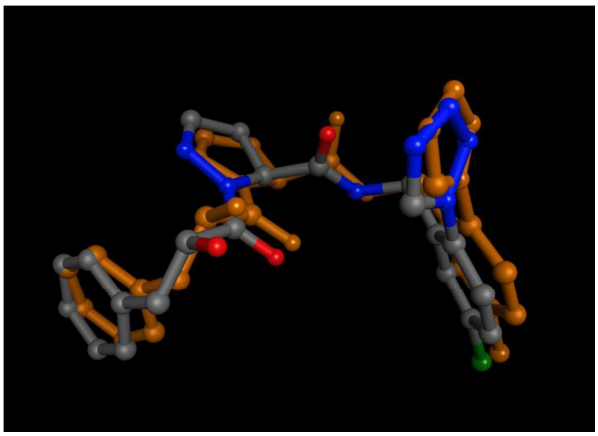


Fig. 5 Overlay of the X-ray structure and the docked structure of compound **55** in the thrombin active site. Docked structure in orange.

functions. As predicted the compounds based on P2 building blocks **20**, **21** and **23** showed only micromolar activity against thrombin. Two compounds that passed the docking filter, containing P2 building blocks **7** and **12**, showed very low activity. However, most of the compounds passing the docking filters also resulted in potent thrombin inhibitors (table 1). Retrospectively, the X-ray structure of compound **55** in complex with thrombin showed good agreement with the docked structure (figure 5). Follow up testing identified five P2 fragments with a promising profile. The pyrazole **13**, the two 3,4-methanoproline isomers **14** and **15** and the two 4,5-methanoproline isomers **17** and **18** all produced compounds with high thrombin potency and high functional in vitro anticoagulant potency in human plasma as measured by the concentration required to double the activated partial thromboplastin time (APTT). The compounds containing these P2's also displayed good Caco-2 cell permeability and good metabolic stability in human liver microsomes (HLM) and showed no hERG or CYP_{3A4} inhibition.[27,28] The 5-oxaproline P2 **6** produced fairly potent compounds with good permeability and stability in liver microsomes but was unstable in human plasma. The pyrazolidine P2 **10** also resulted in potent and permeable compounds but was oxidized in vivo to form the corresponding pyrazole.

Fig. 6 Tolerability studies in mice. The different doses were injected over 30 s only to obtain a very high plasma concentration peak, thereby augmenting the sensitivity of this model. The figures show a comparison between a neutral and a basic compound. Magenta = blood pressure (BP), Blue = heart rate (HR). a. Basic compound. A dose of 40 µmol/kg is not tolerated. b. Neutral compound. A dose of 90 µmol/kg is tolerated.

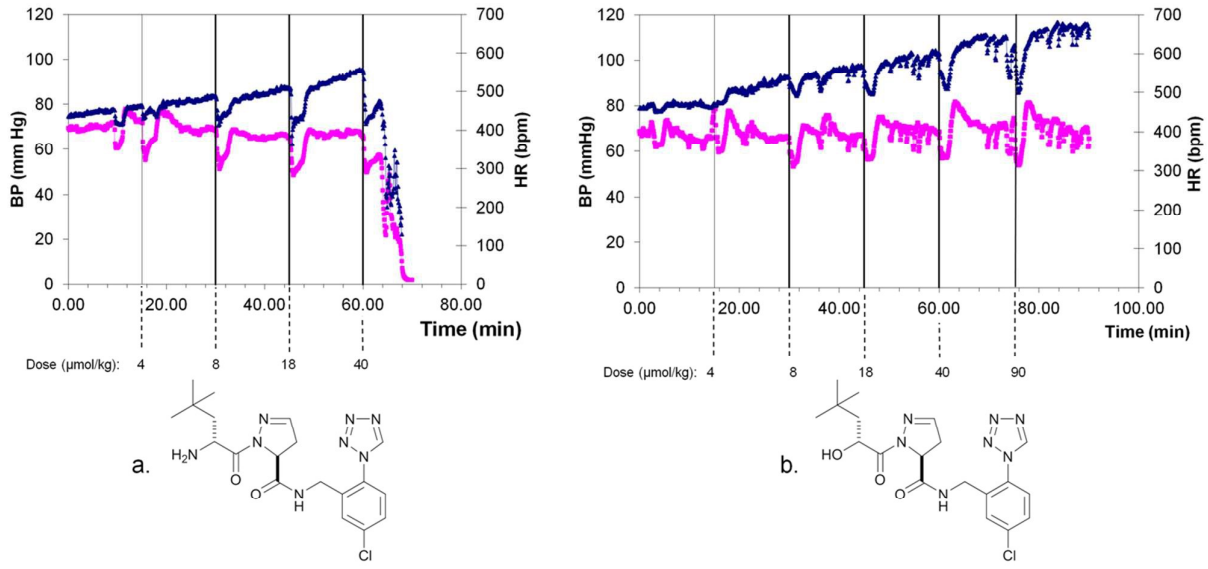
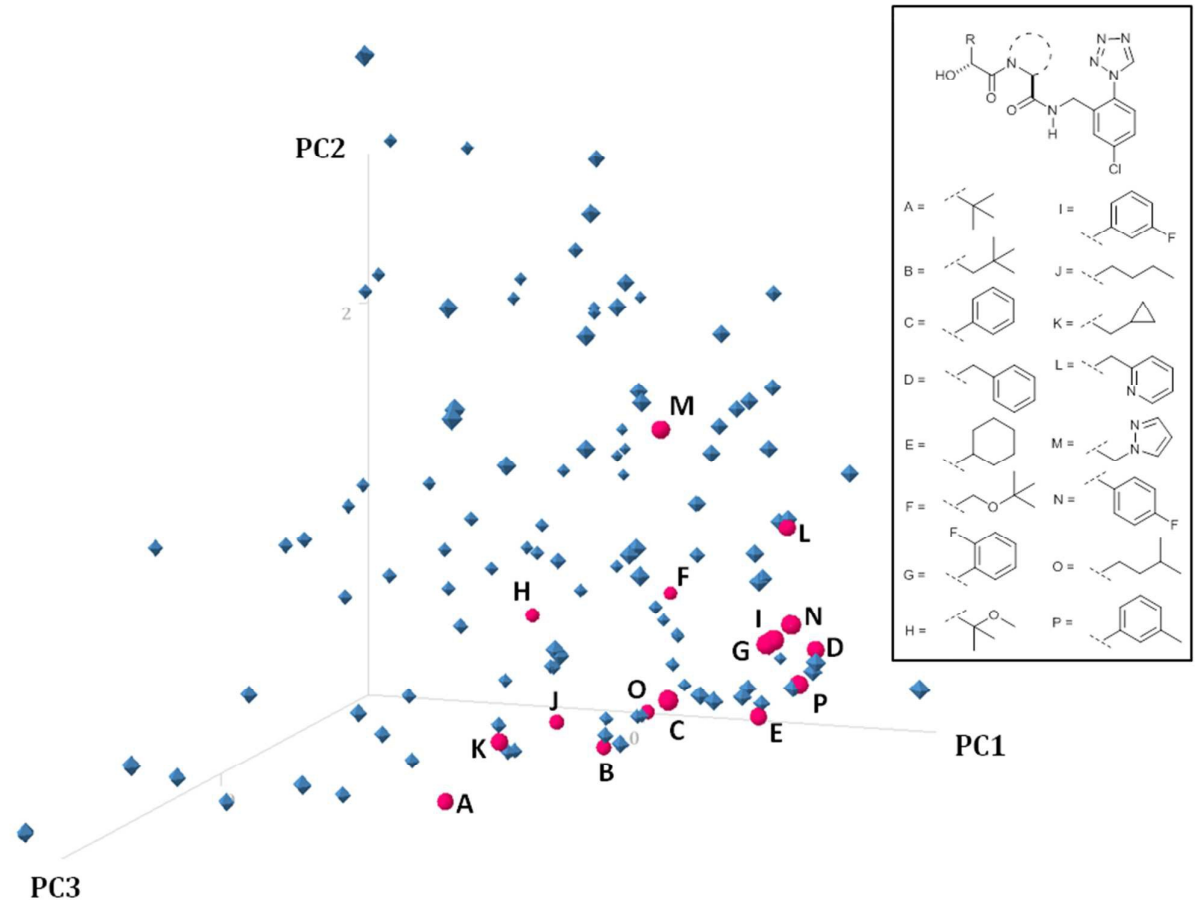


Fig. 7 Selection of compounds for DMTA cycle 2. Plot of principle components (PC) 1-3. PC1 = Parameters related to size. PC2 = Parameters related to polarity. PC3 = Parameters related to flexibility. Red spheres = Compounds selected for synthesis in DMTA cycle 2. R = α-hydroxy-acid side chain (A-P).



DMTA cycle 2 – P3 exploration

In the second design cycle the pyrazole and the methanoproline P2's were combined with the P1 tetrazole and a set of 129 P3 α-hydroxy-acids, selected from both in-house and public thrombin inhibitors, to form a virtual library of 645 compounds. To aid the selection of compounds for synthesis molecular descriptors were calculated and models for Caco-2 cell permeability and metabolic stability in HLM constructed based on the data for the first set of compounds tested.[29] A first filtration step based on docking experiments using hydrogen-bond distance criteria between ligand and Ser-214 and Gly-216 as described earlier (figure 3 and figure 4) was followed by removal of compounds with clogP > 3, predicted Caco-2 P_{app} < 1 and predicted low metabolic stability in HLM. This filtration process reduced the number of P3 α-hydroxy-acids to 77. To visualize and facilitate the selection procedure with the aim to reduce the set of P3 substituents even further a principal component analysis (PCA) was performed of the descriptors for the P3 α-hydroxy-acids.[30] Figure 7 shows the first three principal components where PC1 relates to size, PC2 polarity and PC3 flexibility. Manual inspection of the docking poses was used to aid the selection of α-hydroxy-acids from the clusters. It can be seen that the selected P3 fragments are clustered in an area with high PC1 (larger size) and low PC2 (lower polarity) in line with the fact that the P3 substituents where predicted to bind in the large hydrophobic S3 pocket (D-pocket).[31] This process yielded 10 α- hydroxy-acids selected for synthesis (entry D-M, figure 7). As expected from the nature of the S3 pocket a wide range of substituents is tolerated resulting in very shallow SAR (table 2).

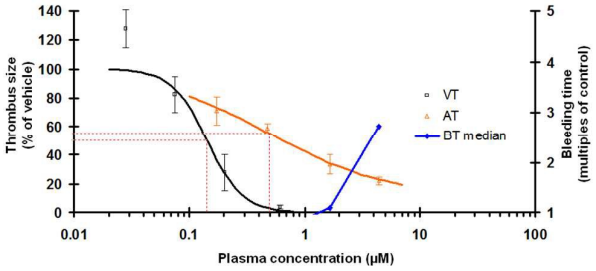


Fig. 8 In vivo efficacy studies of compound **85** in the rat.[32] VT = venous thrombosis, AT = arterial thrombosis, BT = bleeding time.

Journal Name

ARTICLE

Table 3 *In vivo* rat PK and efficacy studies

Compound	APTT IC_{50} (nM, rat)[a]	TCT IC_{50} (nM, rat)[b]	VT IC_{50} (nM)[c]	RLM CL_{int} (µL/min/mg)[d]	Clearance (mL/min/kg, rat)	Bioavailability (%, rat)	CYP_{3A4} IC_{50} (µM)[e]	Solubility (µM)[f]
35	1100	18	120	<15	21	60	28	98
39	820	23	85	<15	18	86	>100	100
42	690	45	150	<15	20	63	>100	97
85	830	13	180	<15	25	75	>50	95
58	540	20	240	<15	38	70	>50	59
44	620	20	66	<15	26	57	47	97

[a] Activated partial thromboplastin time. [b] Thrombin clotting time [c] Venous thrombosis model in the rat.[32] [d] Metabolic stability in rat liver microsomes.[25] [e] Inhibition of human CYP_{3A4} *in vitro*.[27] [f] Solubility in HBSS-MES buffer at pH 6.8.

Testing of this second set of compounds revealed two new P3 substituents with interesting profiles. The butyl and the 2-fluorophenyl substituents produced compounds with high intrinsic thrombin potency and high functional in vitro anticoagulant potency in human plasma as well as good permeability and metabolic stability in human liver microsomes. The compounds showed no hERG or CYP_{3A4} inhibition. Five additional P3s were synthesized based on these results. Compound **84** and **85** (marked O and N in figure 7) were chosen as being close neighbors to the butyl and fluorophenyl P3's in the PCA plot (marked J and I in figure 7). The methylcyclopropylmethyl substituent was chosen as a close analog to the neopentyl substituent. Docking studies indicated that the S3 pocket, around the 3-position on the phenyl-P3, would tolerate small substituents and compound **86**, **87** and **88**, with 3-methyl, 3-cyano and 3-chloro substitution respectively, were synthesized as well but showed no significant advantage (figure 10 and table 2). Compound **88** was very potent with an IC_{50} = 1.9 nM but was as predicted very unstable in human liver microsomes.

Table 4 Selectivity of compound **85** and prodrug **5** towards serine proteases

Enzyme	Compound **5** IC_{50} (µM)	Compound **85** IC_{50} (µM)	Compound **85** Selectivity IC_{50} / IC_{50}
Thrombin	0.15	0.004	-
Trypsin I	86	1.6	400
Trypsin IV		0.41	102
FVIIa		51	12750
FIXa		17	4250
FXa		2.7	675
FXIa		4.6	1150
kallikrein		19	4750
tPA		3.1	775

Biological assessment

A subset of the compounds was tested in the rat showing good functional in vitro potency (APTT) as well as a good in vivo efficacy in a venous thrombosis model (VT).[32] The compounds were stable in rat liver microsomes (RLM) and showed good bioavailability and low to medium clearance in Sprague Dawley rats (table 3). Selected compounds showed no acute toxicity in doses up to 90 µmol/kg in a mouse model where parameters as blood pressure, heart and respiration rate and ECG were recorded (figure 6).[§] Furthermore, the compounds showed

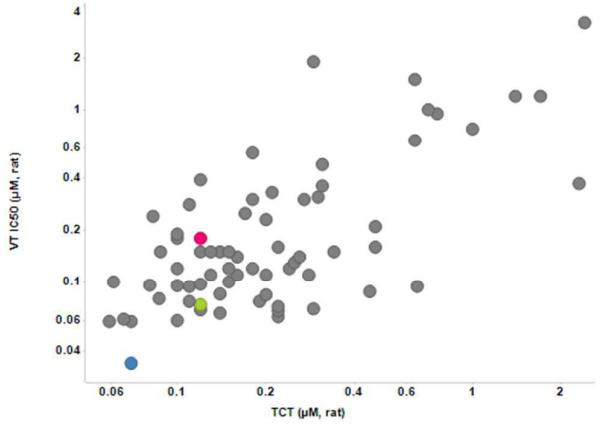


Fig. 9 Correlation between thrombin clotting time (TCT) and effect in a venous thrombosis model (VT) in rat. TCT is defined as the compound concentration that doubles the clotting time. Blue = dabigatran, green = melagatran, red = compound **85**

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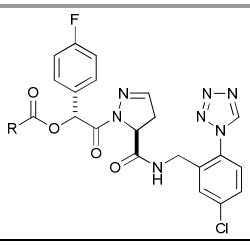
Table 5 Ester prodrugs from compound **85**

Compound **5, 89-92**



Compound	R	Caco-2 P_{app} (10^{-6} cm/s)[a]	Solubility (μM)[b]	CYP_{3A4} IC_{50} (μM)[c]	hERG IC_{50} (μM)[d]	Prodrug hydrolysis (%)[e]	Faeces stability (% remaining/ $t_{½}$ min)
89	Me	30	87.5	>20	>33	48 (51 remaining)	56 / 69
5	Et	33	2.5	8	>33	100	79 / 172
90	Pr	25	<1	1.8	15	83 (11 remaining)	
91	Bu	40	20.5	1.2	14	100	
92	Ph	43			5.6	100	100 / -

[a] Apparent permeability at pH 6.5.[24] [b] Solubility in HBSS-MES buffer at pH 6.8. [c] Inhibition of human CYP_{3A4} *in vitro*.[27] [d] Inhibition of human hERG *in vitro*.[28]

[e] Conversion of prodrug to active inhibitor in human hepatocytes during 2h. [f] Stability in human faeces slurry during 1h.

very low reactivity in a glutathione trapping assay in human liver microsomes. While a five strain Ames test was negative, some of the methanoproline-containing compounds showed mutagenic activity in the presence of S9 fraction in a mouse lymphoma cell thymidine kinase locus assay. However, the pyrazole based compounds showed no mutagenic activity in this assay and the highly potent thrombin inhibitor compound **85** (K_i = 300 pM) was selected for further studies based on its overall superior property profile. In a secondary pharmacology screen containing 101 targets compound **85** was found to bind only to the thromboxane A2 receptor with an IC_{50} of 13.7 μM but showed no antagonistic or agonistic activity in follow-up functional assays. We also examined the selectivity towards other serine proteases. Compound **85** showed a 400x selectivity window against trypsin I, a 675x window against Factor Xa and a >1000x window against Factor VIIa, Factor XIa,kallikrein, plasmin and APC (table 4). As expected, the prodrug **5** showed an even larger selectivity window against trypsin I Compound **85** was tested in prophylactic models of venous and arterial thrombosis in anaesthetized rats (figure 8).The compound was given as a bolus injection, followed by continuous infusion throughout the experiment to obtain a stable plasma concentration profile over time. The plasma concentrations needed to obtain 50% inhibition of thrombus formation were 0.18 and 0.5 μM in the venous and arterial thrombosis models, respectively. In agreement with earlier studies with different thrombin inhibitors the bleeding time in the arterial thrombosis model was prolonged 2-fold when the antithrombotic effect was approximately 70-80%. For all compounds there was a reasonable correlation between thrombin inhibition *in vitro* in rat plasma (thrombin clotting time, TCT) and prevention of venous thrombosis in the rat caval vein thrombosis model (VT, IC_{50}, Fig. 8), illustrating that it is the thrombin inhibition that is responsible for the in vivo effect. Compound **85** showed no acute toxicity in doses up to 90 μmol/kg in a mouse model where parameters as blood pressure, heart and respiration rate and ECG were recorded,

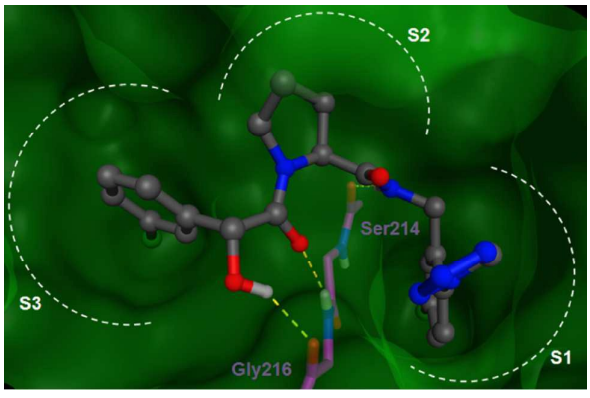


Fig. 10 Docking experiments showing the thrombin active site and a compound with meta-chlorophenyl substitution filling out the S3 pocket. Hydrogen bonds in yellow and protein residues in magenta.

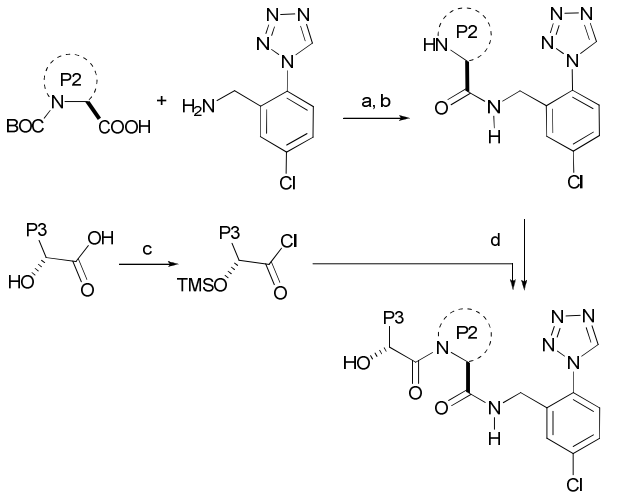
most likely as it has no basic functionality. Plasma protein binding for compound **85** was higher in guinea pig (13 % free) than human (34 % free), dog (26 % free), mouse (33 % free) and rat (36 % free).

Prodrug design and evaluation

Although initial PK studies in rat and dog indicated good PK properties, high-dose PK studies revealed non-linearity and exposure levels of compound **85** in Wistar rats and CD1 mice insufficient for future toxicity studies. Therefore a set of ester prodrugs were synthesized with the aim to increase permeability and exposure levels in the toxicity studies (see table 5). As predicted the ester prodrugs showed high permeability with Caco-2 Papp = 25-43 as compared to the alcohol (Caco-2 Papp = 5.8) and good stability in human intestinal fluid. There were no signs of metabolic reactivity using glutathione trapping for any of the prodrugs. The benzoate **92** showed some hERG inhibition (hERG IC_{50} = 5.6 μM) and the butanoate **90** and pentanoate **91** showed lowmicromolar inhibition of CYP_{3A4}. The propanoate **5** displayed a cleaner conversion to active inhibitor in human hepatocytes as well as better stability in faeces slurry as compared to the acetate **89**. As an added advantage the prodrug **5** displayed a very good selectivity towards trypsin I, a serine protease present in the gut, with a selectivity window of 21500 (table 4). Oral administration of the propanoate **5** resulted in good exposure levels of the active inhibitor compound **85** in rat (F = 64%) and dog (F = 52%) as well as a bioavailability of 30% after colon administration in the rat indicating that an extended release formulation could be possible if needed. The active inhibitor compound **85** was predicted to have a clearance of 2-3 mL/min/kg in man while the corresponding propanoate prodrug **5** was predicted to have a bioavailability in the range of 40-70% with a predicted daily dose in man of 60-150 mg. Data from rat and dog showed that the compound was eliminated both by renal secretion as unchanged (f_e, rat 66%, dog 18%) and by metabolism. Analysis of collected bile from both species indicated that only minute levels of active compound was secreted into the bile. The main CYP involved in the metabolism was CYP_{3A4}. Based on these data we believe that the compound would be eliminated through both metabolism and renal secretion with a predicted variability in exposure (CV% in AUC in an elderly patient population) in the range of 40 %. This approximation can also be compared with a reported variability (CV%) in exposure for dabigatran (thrombin inhibitor) and rivaroxaban (FXa inhibitor) in patients being 69 % and 50-55 %, respectively.[33,34] Both the active compound **85** and the prodrug compound **5** displayed a good exposure margin in safety pharmacology studies including inhibition of hERG (>94-fold margin) and other cardiac ion channels (>90-fold margin to hCav1.2, hCav3.2, hKv1.5, hNAv1.5, hHCN4 and hKvLQT1-hminK and >50-fold margin to hKV4.3-hKChIP2.2), cardiac electrophysiology studies in rat, mouse and guinea pig (>40-fold margin), an Irwin study in the rat (>24-fold margin) as well as in *in vivo* toxicology studies in rat and dog as compared to the projected free C_{max} in human. In a phase I trial in healthy male volunteers, compound **5** was found to be safe and well tolerated following an oral dose of 2.5 mg.[§§] This was an explorative study allowing only low exposure and pharmacological response. The stopping criterion for pharmacological response (mean prolongation of thrombin clotting time (TCT) > 10 sec) was achieved already at the 2.5 mg oral dose. The pharmacokinetic profile was essentially as predicted with rapid bioconversion of the prodrug and low variability. No prodrug was detected in samples of plasma or urine. The active form had a relatively short half-life of about 2 hours and the coefficient of variation of the plasma exposure (AUC) was 21%, which compares well to the coefficient of variation of dabigatran etexilate of 44 % in healthy volunteers.[33] Pharmacologic response was shown to be correlated to plasma concentration for the ex vivo coagulation time assay TCT and ecarin clotting time (ECT). The results supported a therapeutic dose of about 100 mg just as predicted based on preclinical data.

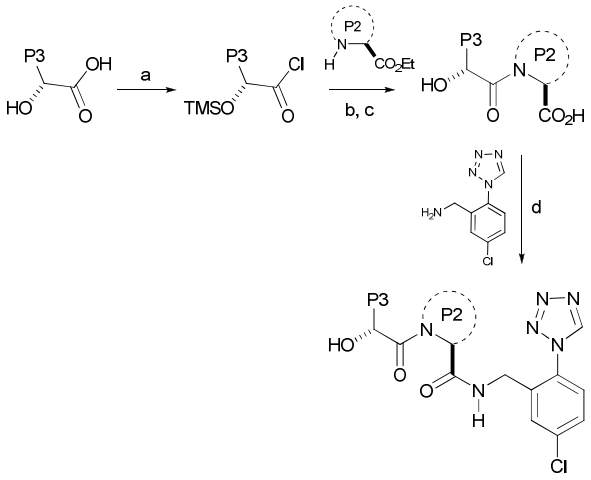
Chemistry

The compounds described in this article were synthesised using the methods depicted in scheme 1 and 2.[19,20] For analytical data see the supplementary information.



Scheme 1. Synthesis of compounds containing P2 building blocks **6-12** and **14-23**. Reagents and conditions:

(a) HOBt, EDC, TEA, CH_2Cl_2, r.t.; (b) HCl (conc., aq.), MeOH, r.t.; (c) TMSCl, DMAP, pyridine, r.t., then DMF (cat.), CO_2Cl_2, CH_2Cl_2, 0 °C to r.t.; (d) pyridine, CH_2Cl_2, r.t., then MeOH.



Scheme 2. Synthesis of compounds containing P2 building block **13**. Reagents and conditions:

(a) TMSCl, DMAP, pyridine, r.t., then DMF (cat.), CO_2Cl_2, CH_2Cl_2, 0 °C to r.t.; (b) pyridine, CH_2Cl_2, r.t., then MeOH; (c) LiBr, Et_3N, CH_3CN r.t.; (d) NMM, TBTU, EtOAc, r.t.

Conclusions

We have described a highly effective selection process based on docking experiments and property calculations and predictions. This strategy resulted in the identification of the highly potent (K_i = 300 pM) thrombin inhibitor compound **85** and the corresponding propionate prodrug AZD8165 as a candidate for clinical evaluation in the treatment of thrombosis and related diseases. AZD8165 was found to be safe and well tolerated and delivered a positive Proof of Principle in a phase I trial in healthy male volunteers.§§§

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Notes and references

‡ Calculated ACD $logD_{7.4}$ for the P1 fragments: 1,4-dichloro-2-methyl-benzene (logD = 4.0), 2-(4-chloro-2-methyl-phenoxy)-N-ethyl-acetamide (logD = 3.0), 1-(4-chloro-2-methyl-phenyl)tetrazole (logD =2.0)

§ All animal experiments were approved by the Local Ethics Committee on Animal Experiments in Göteborg, Sweden. Protocol number 183-2005 and and 9-2006

§§ The clinical study was approved by the independent Institutional Review Board (IRB)/Independent Ethics Committee (IEC), Midlands Institutional Review Board (8417 Santa Fe Drive, Suite 100, Overland Park, Kansas, United States). The study was performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with ICH/Good Clinical Practice and applicable regulatory requirements and the AstraZeneca policy on bioethics. Study code D2890C00001

§§§ Compound **85** (AZ12971554) is available through the AstraZeneca open innovation program - **http://openinnovation.astrazeneca.com/what-weoffer/compound/az12971554/**

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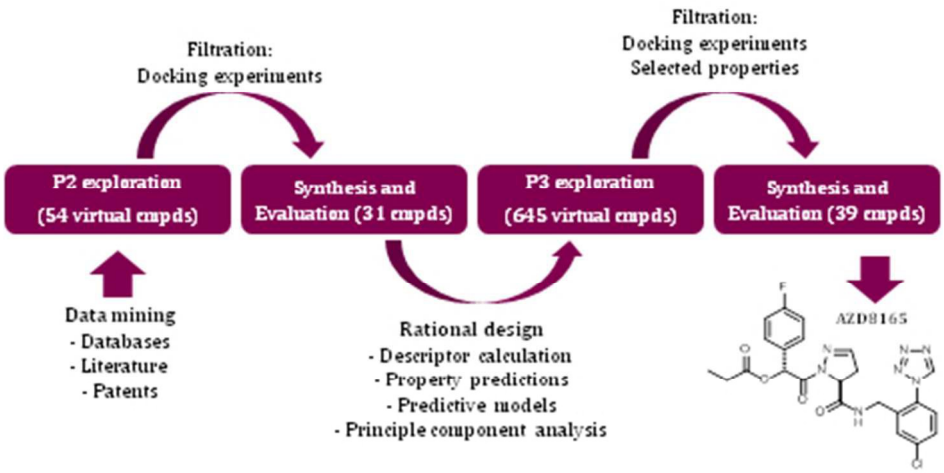

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