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Novel factor Xa inhibitors based on a benzoic acid scaffold and incorporating a neutral P1 ligand

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Abstract—A series of novel, highly potent, achiral factor Xa inhibitors based on a benzoic acid scaffold and containing a chlorophenethyl moiety directed towards the protease S1 pocket is described. A number of structural features, such as the requirements of the P1, P4 and ester-binding pocket ligands were explored with respect to inhibition of factor Xa. Compound **46** was found to be the most potent compound in a series of antithrombotic secondary assays.

1. Introduction

Currently available drug treatment for thrombosis-related diseases is less than satisfactory and fraught with difficulties such as severe side effects requiring monitoring of drug levels, slow onset of action, mode of administration and potentially life-threatening bleeding.[1] The trypsin-like serine protease factor Xa is an attractive target for antithrombotic therapy as it occupies a unique position at the convergence of the intrinsic and extrinsic pathways,[2] and thus affects coagulation without directly affecting platelet function. A huge amount of effort has been invested in the search for an orally available factor Xa inhibitor, which should overcome many of the disadvantages of current therapies. Although many highly potent and selective inhibitors of factor Xa have been discovered, their major shortcoming has often been the presence of highly basic benzamidine or guanidine moieties used as arginine mimetics, and designed to bind in the P1 position. These moieties generally result in extremely poor oral bioavailability of the inhibitors in which they are incorporated.[3] Here we report the synthesis of novel, potent and highly selective factor Xa inhibitors, which contain neither a benzamidine nor a guanidine moiety, and incorporate a neutral chlorophenyl P1 ligand.

Synthesis of a directed library targeting factor Xa led to the discovery of compound **1**, with a K_i of 153 nM[4] (Fig. 1). This compound was initially expected to bind with the guanidino group in the S1 pocket, and the dichlorophenethyl group in the S4 pocket. However the X-ray crystal structure of factor Xa crystals with this ligand showed clearly that **1** bound in the active site of factor Xa with the guanidino group in the S4 pocket, the methoxy group in the 'ester-binding pocket' (EBP)[5], which is a binding site subpocket adjacent to S1, and the dichlorophenethyl group in the S1 pocket, whereby the *para* chloro substituent displaced a water molecule normally bound in the base of the S1 pocket. (Results will be published elsewhere.) This 'chloro-binding mode' has also been discovered independently by other researchers.[6] Synthesis of analogues of **1**, aimed at replacing the arginine amide by simpler, smaller and more rigid moieties gave compound **2**, with a K_i of 189 nM. This compound was the starting point in the current investigation.[7]

2. Non-guanidino/amidino P4 ligands

Scheme 1 outlines the synthesis of a small library of analogues of **2**. Compound **4** was synthesized in

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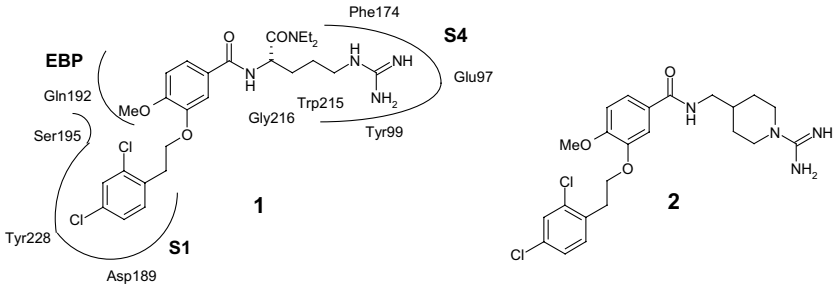
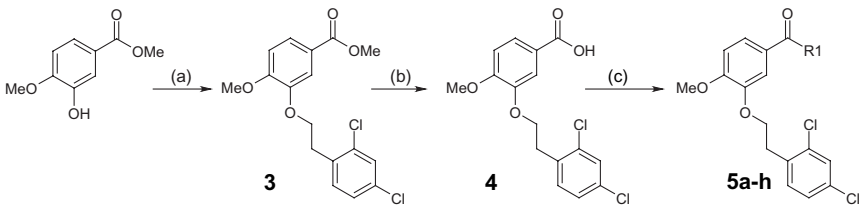


Figure 1. Schematic diagram showing relative positions of S1, S4 and ester-binding pockets and the binding mode of this class of factor Xa inhibitors.



Scheme 1. Reagents and conditions: (a) 2-(2,4-dichlorophenyl)-ethanol, DEAD, PPh_3–polystyrene, THF/rt, 16 h; (b) (i) NaOH (aq), dioxan/60 °C, 1 h, (ii) HCl/water, precipitation; (c) amine, TOTU, *N*-ethylmorpholine, DMF.

multi-gram amounts starting from commercially available 3-hydroxy-4-methoxy-benzoic acid methyl ester. Analogous procedures were used to synthesize all other compounds described herein, typically on a 50 mg scale. The Mitsunobu alkylation gave yields typically of between 40% and 95%. Saponification proceeded in near quantitative yield. The final amide coupling gave yields of between 20% and 90%, depending on the combination of building blocks. Using an in-house synthesis robot a library of 330 amides of structure **5** was synthesized, using amines which were pre-selected for fit into the S4 pocket. The eight most active compounds, with K_i values less than 1 μM are shown in Table 1.

Compound **5a** was by far the most active factor Xa inhibitor in this series, and an order of magnitude more active than lead compound **2**. With the exception of compound **5e**, the other active compounds found (**5b**–**h**) were all minimally modified analogues of **5a**, and all considerably less active. Compound **5e** was found to be strongly metabolized by N-oxide formation on incubation with S9 liver fraction, and was not considered for further investigation. Compound **5a** was selected as the starting point for the optimization of the P1 and EBP binding ligands.

3. Structural requirements of the P1 ligand

As a first step in our investigation of P1 ligands, we chose to examine the role of the chlorine in the 2-position of the dichlorophenethyl group. This substituent is not involved in the displacement of water.

We simultaneously modified the EBP ligand since the proximity of the S1 pocket and the EBP opens up the possibility of interaction between the EBP ligand and the 2-substituent of the S1 binder. The results are shown in Table 2. The compounds were synthesized analogously to compound **5a** using different alcohols for the Mitsunobu alkylation of the phenol of the central scaffold.

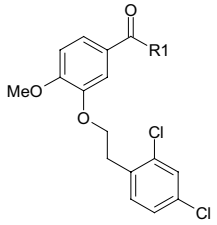
Compounds **5a**–**13** were also tested for their inhibition of thrombin[4] and in all cases had a K_i of >2.5 μM.

Clearly the 2-chloro substituent is important for potency in the case where the EBP ligand is methoxy. Removal of the chlorine substituent results in a 2-fold drop in potency (**6**) and introduction of a pyridyl nitrogen at the 2-position (**7**) results in a 3-fold drop in potency. This trend is essentially reversed in the case where the EBP ligand is chlorine (compounds **8**–**10**). Here the removal of the 2-chloro substituent of the P1 ligand results in a 2–3-fold increase in potency.

A slight improvement in potency is also observed when the EBP ligand is fluorine. These findings confirm that there is indeed interaction of the EBP ligand and the substituent at the 2-position of the chlorophenethyl P1 ligand. When the EBP ligand is a halogen then the 2-chloro P1 substituent, which is critical for potency in compound **5a**, is unfavourable.

We next turned our attention to replacement of the 4-chloro substituent of the P1 ligand. This moiety is critical for driving the binding of these inhibitors to factor Xa as it displaces a bound water molecule at the base of the S1 pocket. The results in Table 2 showed that for monosubstituted P1 ligands more potent factor Xa inhibitors are obtained when a halogen is selected as the EBP ligand. We thus elected to synthesize and test inhibitors with a halogen in the EBP combined with a selection of monosubstituted P1 ligands. The results are shown in Table 3. Clearly a *para* chloro substituent

Table 1. Benzoic acid-based factor Xa inhibitors with K_i values <1 μM from directed library of non-guanidino/amidino P4 ligands[4]



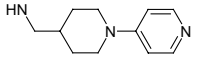
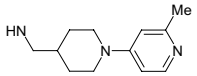
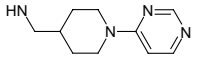
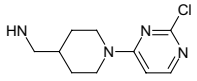
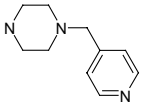
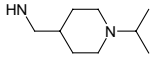
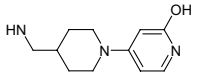
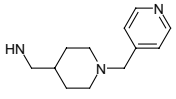
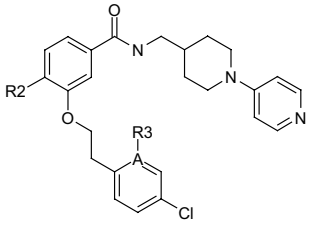
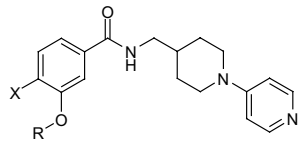
Compound	R1	K_i (FXa)/nM
5a		18
5b		57
5c		110
5d		264
5e		600
5f		650
5g		950
5h		970

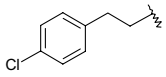
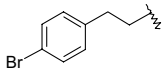
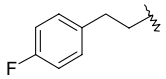
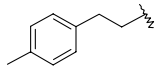
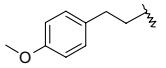
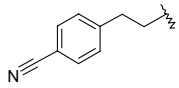
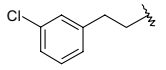
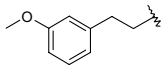
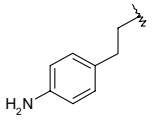
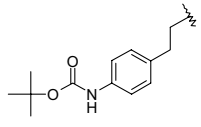
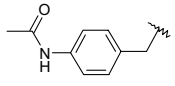
Table 2. Simultaneous modification of the 2-position of the P1 ligand and the EBP ligand



Compound	R2	R3	A	K_i (FXa)/nM
5a	OMe	Cl	C	18
6	OMe	H	C	41
7	OMe	—	N	54
8	Cl	Cl	C	41
9	Cl	H	C	13
10	Cl	—	N	20
11	F	Cl	C	50
12	F	H	C	40
13	F	—	N	37

Table 3. SAR of water-displacement moiety in P1 ligand



Compound		R	K_i (FXa)/nM	
X = Cl	X = F		X = Cl	X = F
9	**12**		13	40
14	**24**		36	71
15	**25**		102	203
16	**26**		124	206
17	**27**		314	307
18	**28**		1552	4605
19	**29**		1931	712
20	**30**		2494	3348
21	**31**		420	1554
22	**32**		4531	>10,000
23	**33**		>10,000	>10,000

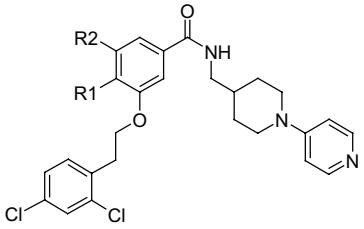
(**9** and **12**) is far superior to all other substituents. For example, in compounds **14**–**16** bromo and fluoro *para* substituents, as well as a *para* methyl substituent also retain nanomolar activity, but are approximately 3-, 8- and 9-times less potent, respectively, than compound **9**. Compounds **19** and **29**, the *meta* chloro analogues of compounds **9** and **12** are very much less potent than the *para* chloro parent compounds. Interestingly, methoxy substituents (**17**, **27**, **20**, **30**) are considerably less potent

than halogen or methyl substituents. The use of methoxy as an amidine replacement has previously been reported.[8]

4. Structural requirements of the EBP ligand

Table 4 shows results for inhibitors with variation of the EBP ligands combined with the dichlorophenethyl P1 ligand. The series **34**, **11**, **8**, **35** and **36**, with increasing size of the R1 substituent, indicates that of the halogen substituents chlorine has the optimal size in this position. Bromo and iodo substituents are too large, and fluoro and H substituents too small. Other small substituents like methyl (**37**) and nitrile (**39**) are also potent, but trifluoromethyl (**38**) is already too large. The series **40**–**45** show that a simple amino substituent (**40**) is more potent than chloro. Systematically increasing the size of the substituent by alkylation or acylation of the amino function results in a reduction in potency (**41**–**44**), as does reversing the amido function (**45**). Interestingly, shifting the $CONH_2$ substituent to position R2 in compound **46** results in a 10-fold increase in potency compared to compound **45**. The series **5a**, **47**–**49** shows the deleterious effect of introducing larger analogues of methoxy. Shifting the methoxy substituent to position R2 (**50**) also results in a loss of potency, which can be partially recovered by introducing a second methoxy group at R1 (**51**). Compound **52** with two ethoxy substituents is considerably reduced in potency. Bridging positions R1 and R2 through a methylene dioxy ring, gave compound **53**, which is also less potent than **5a**.

Table 4. Structure–activity relationships in the ester-binding pocket



Compound	R1	R2	K_i (FXa)/nM
34	H	H	106
11	F	H	50
8	Cl	H	41
35	Br	H	76
36	I	H	156
37	CH_3	H	37
38	CF_3	H	140
39	CN	H	44
40	NH_2	H	29
41	$NHCH_3$	H	48
42	NHCOH	H	75
43	$NHCOCH_3$	H	316
44	$NHCONH_2$	H	1763
45	$CONH_2$	H	588
46	H	$CONH_2$	51
5a	CH_3O	H	18
47	CH_3S	H	28
48	CH_3CH_2O	H	61
49	*i*PrO	H	233
50	H	CH_3O	125
51	CH_3O	CH_3O	50
52	CH_3CH_2O	CH_3CH_2O	263
53	OCH_2O		39
54	Br	CH_3O	20
55	Br	CH_3CH_2O	86
56	CH_3O	Br	1053
57	Cl	CH_3O	10
58	CH_3	CH_3O	13

Compounds **54**–**57** show the effect of combining alkoxy substituents with halogens in the EBP. Compound **57**, as well as compound **58** with a methyl group in R1 and a methoxy group in R2, are the most potent factor Xa inhibitors in this series.

5. Secondary assays

The anticoagulant effect of factor Xa inhibitors was determined in citrated human plasma spiked with increasing concentrations of inhibitor. The standard coagulation assays activated partial thromboplastin time (APTT) and prothrombin time (PT) were measured, as well as the more sensitive dilute PT (dPT) with diluted thromboplastin reagent.[4] Surprisingly, compound **46**, which in the enzyme assay is approximately 2 and 5 times less potent than **5a** and **57**, respectively, is around 2 and 5 times more potent than these compounds in the more stringent and predictive dPT antithrombotic assay, as well as being more potent in the APTT and PT assays[4] (Table 5). The reason for this trend is not clear, especially since all three compounds show similar plasma protein binding. In addition, **46** has a predicted 100% intestinal absorption in the Caco-2 assay, as well as good stability in human S9 liver fraction.

In conclusion, we have discovered and optimized a new class of potent non-guanidino/amidino factor Xa inhibitors, based on a simple achiral benzoic acid scaffold. Many of the compounds are predicted to be orally bioavailable and metabolically stable. The compounds are capable of reducing the dilute prothrombin time, a

Table 5. Antithrombotic, predicted absorption and metabolic stability of selected compounds

Compound	K_i (FXa)/nM	dPT/nM	APTT/μM	PT/μM	Caco-2[a] P_{app}/nm s^{-1}	S9[b]/%
5a	18	237	7.30	3.66	47.8	96
57	10	521	14.35	6.95	0.51	86
46	51	111	2.86	2.29	12.4	90

[a] Apparent permeability coefficient.
[b] Percent compound remaining after 2 h incubation with human S9 liver fraction.

recognized surrogate parameter for in vivo antithrombotic activity in the clinic, and are therefore promising drug substances for the treatment of thrombotic diseases.

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