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Factor Xa inhibitors based on a 2-carboxyindole scaffold: SAR of neutral P1 substituents

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Abstract—A series of novel, highly potent 2-carboxyindole-based factor Xa inhibitors is described. Structural requirements for neutral ligands, which bind in the S1 pocket of factor Xa were investigated with the 2-carboxyindole scaffold. This privileged fragment assembly approach yielded a set of equipotent, selective inhibitors with structurally diverse neutral P1 substituents.

1. Introduction

The currently available anticoagulants for the treatment and prevention of thromboembolic diseases including deep vein thrombosis, myocardial infarction and pulmonary embolism are handicapped by several shortcomings, including slow onset of action, mode of administration and severe, potentially life-threatening side effects like excessive bleeding requiring stringent monitoring of drug-levels.[1] The serine protease factor Xa (fXa) has emerged as an attractive target for the therapy of thrombosis-related diseases since it is a key enzyme in the activation cascade of the coagulation system linking the extrinsic and intrinsic activation pathways.[2] It is anticipated that inhibition of factor Xa should prevent thrombus formation without compromising normal hemostasis and platelet function.[3] An orally available fXa inhibitor should therefore be a superior antithrombotic agent overcoming the shortcomings associated with the current treatment. A major obstacle to the development of an orally available drug from the many potent and selective inhibitors available is often the need to incorporate a highly basic benzamidine or guanidine serving as an arginine mimetic in the S1 pocket of fXa. In general these moieties are linked to the extremely poor bioavailability observed for the inhibitors bearing this functionality.[4] Here we report the synthesis of novel, potent and selective fXa inhibitors based on a 2-carboxyindole scaffold, which contain neither a benzamidine nor a guanidine moiety, and incorporate a variety of neutral P1 ligands directed towards the fXa S1 pocket.

Figure 1 gives a schematic representation of the design rationale for the 2-carboxyindole fXa inhibitors. In a previous communication[5] we described the optimization of a benzoic acid scaffold incorporating a neutral P1 ligand, which interacts in the S1 pocket via the so-called chloro binding mode.[6] We succeeded in reaching low nanomolar activity against fXa. However, concurrently we were intrigued by the additional opportunities the identification of alternative scaffolds with different topology to the 1,3 connectivity of the P1 and P4 groups of the benzoic acid series would present. We suspected that modification of both the central scaffold and the connectivity pattern might allow us to incorporate different S1 and S4 directed ligands, and thus open alternative routes to optimize physicochemical and pharmacological profiles while maintaining fXa binding affinity. In addition the recently described[7] successful use of ketopiperazines as scaffolds with a 1,4-connective relationship of P1 and P4 substituents indicated that the investigation of alternative connectivities could be a promising approach. We decided, therefore, to investigate N-alkylated 2-indolecarboxamides as nonchiral,

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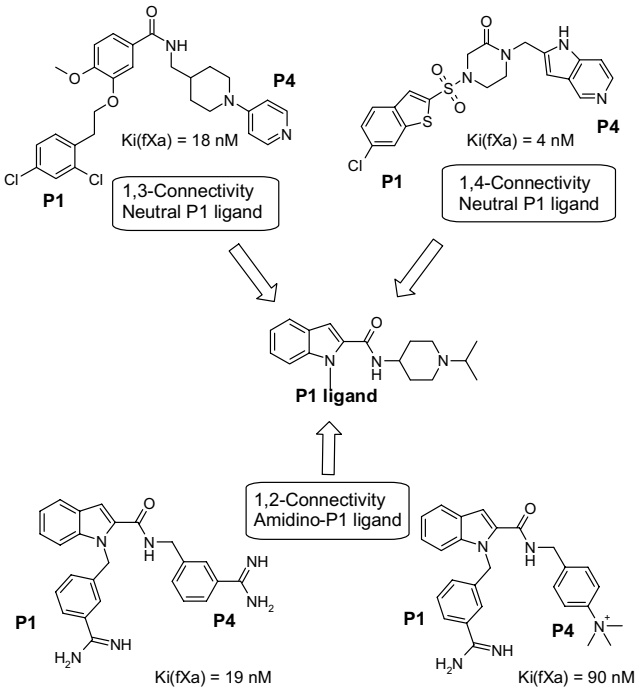


Figure 1. Rationale for the design of 2-carboxyindole factor Xa inhibitors with neutral P1 ligands.

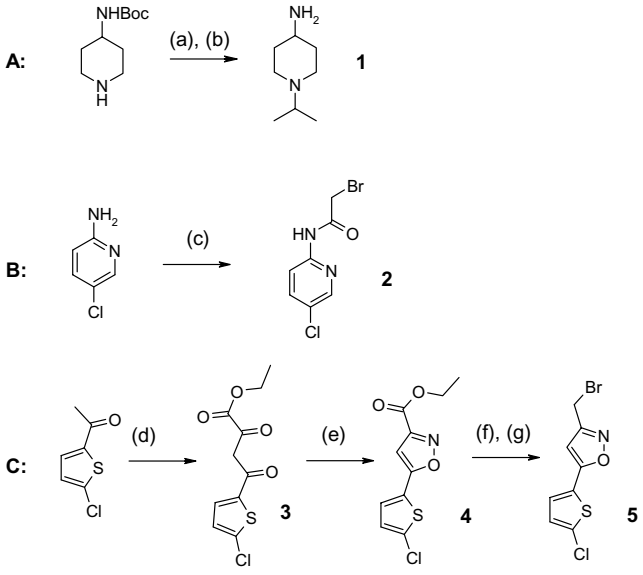
rigid α-amino acid mimetics offering an alternative and potentially superior trajectorial orientation for the P1/P4 ligands. This scaffold had previously been reported in combination with less favourable benzamidine S1 ligands.[8] In addition, in terms of library generation the chemistry for the decoration of 2-carboxyindoles has the advantage of being very flexible, enabling the attachment of the respective ligands first at the indole nitrogen or alternatively at the carboxy group.

Here we describe 2-carboxyindole derivatives as fXa inhibitors, with special emphasis on the SAR of the P1 ligand.

2. Chemistry

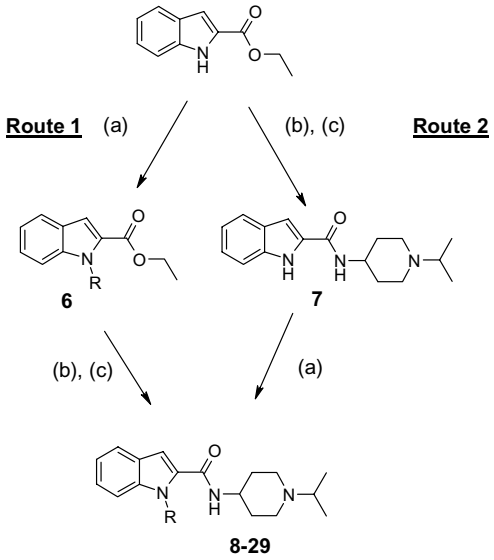
Scheme 1 shows the synthesis of representative ligand building blocks, which were subsequently attached to the 2-carboxyindole scaffold. The P4 ligand **1** used throughout was prepared from commercially available *N*-Boc protected 4-aminopiperidine in two steps by reductive amination with acetone followed by acid catalyzed removal of the Boc group. Bromoacetamide **2** was prepared in one step by acylation of 2-amino-5-chloro pyridine with bromoacetyl bromide. The biaryl bromide **5** was synthesized starting from 1-(5-chlorothiophen-2-yl)-ethanone by condensation with oxalic acid diethyl ester followed by regioselective formation of the isoxazole ring by treatment with hydroxylamine. Reduction of the ester in **4** and subsequent bromination yielded bromide **5**.

The synthesis of the fully decorated indole scaffold with the various putative P1 ligands was carried out by two optional routes starting from commercially available



Scheme 1. Reagents and conditions: (a) acetone, $NaBH_3CN$, AcOH, methanol, rt, 16 h; (b) HCl/MeOH (8 M), rt, 16 h; (c) $BrCH_2COBr$, pyridine, toluene, rt, 2 h; (d) KOtBu, then oxalic acid diethyl ester, toluene, rt 3 h; (e) $NH_2OH{\cdot}HCl$, ethanol, reflux, 6 h; (f) $NaBH_4$, ethanol, 0 °C, 3 h; (g) NBS, PPh_3, CH_2Cl_2, rt, 1 h.

indole-2-carboxylic acid ethyl ester (Scheme 2). N-arylated compounds **8**–**10** were synthesized according to route 1 by $Cu(OAc)_2$ mediated coupling of aryl boronic acids to the indole nitrogen.[9] The N-alkylated precursors were synthesized under standard conditions using the corresponding bromides or tosylates in the presence of NaH in DMF. Saponification of the ester **6** was followed by amide coupling of the acid with isopropyl-



Scheme 2. Reagents and conditions: (a) $ArB(OH)_2$, $Cu(OAc)_2$, pyridine, NEt_3, CH_2Cl_2, 50 °C, 5 days; or Ar–(CH_2)–Hal, NaH, DMF, 80 °C, 1 h; or Ar–$(CH_2)_2$–OTos, NaH, DMF, rt, 16 h; or Ar–$NHCO(CH_2)$–Br, NaH, DMF, rt, 2 h; (b) LiOH, THF/H_2O, 60 °C, 2 h; (c) amine **1**, TOTU, *N*-ethylmorpholine, CH_2Cl_2; or BOP–Cl, NEt_3, CH_2Cl_2.

piperidine **1** using either TOTU or BOP–Cl in CH_2Cl_2 to yield the final derivatives **8**–**29**. Route 2 essentially postponed the diversifying indole N-alkylation step to the end of the sequence and was successfully applied for the synthesis of compounds **26**–**29** (Table 3). This synthetic route proved to be superior for the preparation of compounds incorporating the *N*-acetamide linker, which turned out to be rather unstable under the basic or acidic conditions required for ester cleavage. In addition the use of route 2 prevented cyclization to the six membered *N*-aryl-indolo-1,3-ketopiperazine, which occurred to a considerable extend under the acyl activation conditions used in route 1.

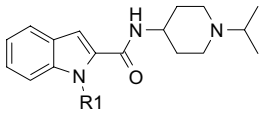
3. Results and discussion

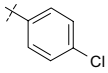
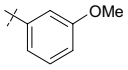
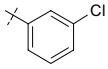
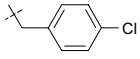
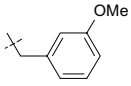
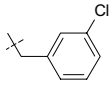
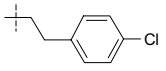
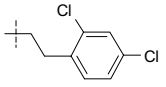
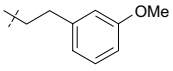
The use of a scaffold providing new trajectories into the S1 and S4 pocket required a review of privileged P4 ligands (i.e., fragments known to impart good potency) under the stipulation that the neutral P1 ligand should be attached at the indole nitrogen. A solution phase library of 140 compounds containing 14 prototypic conformationally rather mobile P1 ligands and 10 potential P4 ligands at the indole scaffold (results not shown) revealed *N*-isopropyl-4-aminopiperidine as a highly potent P4 ligand. This group was selected for the present SAR studies on the P1 substituents and was incorporated in all the inhibitors described in this communication.

Table 1 shows the SAR for a series of simple, monocyclic P1 groups, with varying lengths of spacer. Clearly the directly arylated inhibitors **8**–**10** were too short for close interaction with Tyr 228 at the base of the S1 pocket,[6] although some nuances in activity were evident. In contrast to the equipotent *para*-chlorophenyl compound **8** and the *meta*-methoxyphenyl derivative **9**, the *meta*-chlorophenyl compound **10** was much less active. A different pattern of activity was observed for compounds **11**–**13** with a methylene spacer connecting the P1 ligand with the scaffold. *para*-Chlorobenzyl compound **11** was the least potent in this series. *meta*-Chlorobenzyl **13**, showed intermediate potency and, remarkably, the *meta*-methoxybenzyl compound **12**, had a K_i value under 100 nM,[10] indicating a strong and specific interaction in the S1 pocket. Substituted phenethyl ligands, which we previously used successfully in combination with a benzoic acid scaffold,[5] were incorporated in compounds **14**–**16**. The *para*-chlorophenylethyl moiety in compound **14** exhibited the most promising activity as P1 ligand in this context. The dichloro derivative **15** was approximately 6-fold less potent than **14** and the *meta*-methoxyphenylethyl substitution (**16**) was even less potent.

The results in Table 1 established that the structural requirements for the P1 ligand in the most potent inhibitor (**14**) were a terminal chloro substituent combined with an extended spacer. We next turned our attention to the optimization of this rather conformationally mobile P1 ligand, combining further extension of the linker length with the required rigidification

Table 1. 2-Carboxyindole-based factor Xa inhibitors incorporating simple P1 ligands

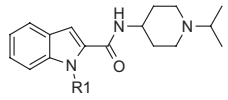


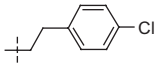
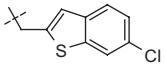
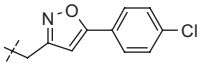
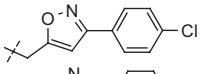
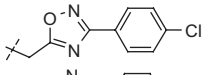
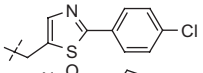
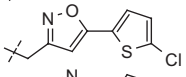
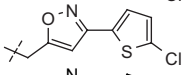
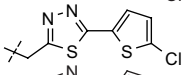
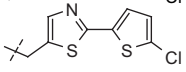
Compds	R1	K_i (fXa) nM
8		2100
9		2480
10		9010
11		2500
12		89
13		654
14		73
15		474
16		>10,000

(Table 2). A chlorobenzothiophene P1 ligand in **17** did indeed improve potency compared to **14**. Surprisingly extension to a biarylic system resulted in a rather dramatic fall in activity (**18**–**21**), indicating the sensitivity of the interaction between the chloroaryl fragment and Tyr 228 to changes in the trajectory and spacing of the P1 ligand. Moreover, a more detailed analysis revealed the critical role played by the first heterocyclic ring and its polarity distribution. Isoxazole **18** was only half as active as the reversed derivative **19**. Compounds **20** and **21** incorporating a oxadiazole and thiazole ring, respectively, were even less active suggesting that distinct polar interactions were required at one side of the ring whereas such interactions at the other side of the ring were deleterious. This generally disappointing loss in activity on rigidifying the ethyl spacer of the *para*-chlorophenyl ring could be more than completely reversed in the biaryl series by replacement of the *para*-chlorophenyl moiety by chlorothiophene (**22**–**25**). For example, exchange of the *para*-chlorophenyl group in **18** for a chlorothienyl group in **22** resulted in a dramatic 270-fold increase in activity.

Apparently this was due to the slight trajectorial change in the presentation of the chlorine (essential for binding) on shifting from *para*-chlorophenyl to its chlorothienyl analogue. This gain in activity can be attributed to an optimal positioning of the chlorothiophene enabling a close contact of the chlorine atom with the centroid of

Table 2. 2-Carboxyindole-based factor Xa inhibitors incorporating extended and rigidified P1 ligands

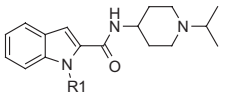


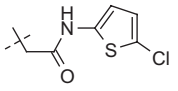
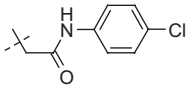
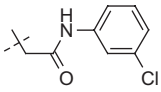
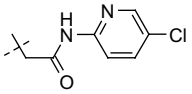
Compds	R1	K_i (fXa) nM
14		73
17		40
18		810
19		400
20		2230
21		2730
22		3
23		3
24		4
25		57

the aromatic ring of Tyr 228 located at the base of the S1 pocket, as described previously.[6] Although the impact of this interaction masked the more subtle differences in interaction between the first isoxazole ring in compound **22** and the reversed isoxazole **23** or the thiadiazole derivative **24** the presence of a less polar thiazole ring in compound **25** was still deleterious, resulting in a 19-fold loss in activity.

The final type of rigidified P1 ligand investigated was the acetamides (**26**–**29**) shown in Table 3. These are of

Table 3. 2-Carboxyindole-based factor Xa inhibitors incorporating acetamide rigidified P1 ligands



Compds	R1	K_i (fXa) nM
26		15
27		3
28		287
29		1

intermediate length to the flexible ethyl spacer in compound **14**, and the biaryl derivatives **18**–**25**. In this series the acetamide-linked chlorothiophene in **26** proved still to be considerably active, but in contrast to the biaryl series the activity of the *para*-chlorophenyl analogue **27** surpassed that of all other *para*-chlorophenyl derivatives synthesized. Altering the trajectory of the chlorine atom in the *meta*-chlorophenyl derivative **28** resulted in a considerable drop in activity. However, potency could be increased by introducing a nitrogen resulting in the most potent inhibitor (**29**) in this series ($K_i = 1$ nM). This outcome again reflects that in conjunction with the chloro binding mode, polar interactions along the S1 pocket of the fXa enzyme can influence the affinity of inhibitors with neutral P1 ligands.

Table 4 shows the activity of key compounds (**17**, **22**, **27**, **29**) against related proteases and confirms the high selectivity of the compounds in this series. These compounds were evaluated (Table 5) for their anticoagulant

Table 4. Selectivity profile for selected 2-carboxyindole-based factor Xa inhibitors

Compds	fXa K_i/μM	Thrombin K_i/μM	Trypsin K_i/μM	Kallikrein K_i/μM	t-PA[a] K_i/μM
17	0.040	9.04	>100	>100	>100
22	0.003	2.76	>100	15.4	>100
27	0.003	>10	>100	>100	>100
29	0.001	>10	>100	>100	>100

[a] Human tissue plasminogen activator.

Table 5. Anti-fXa, anticoagulant activity, predicted absorption and metabolic stability of selected compounds

Compds	fXa K_i/μM	dPT[a]/μM	APTT[b]/μM	PT[b]/μM	S9[c] %	P_{app}[d] nm/s
17	0.040	0.897	17.04	14.13	98	1.5
22	0.003	0.426	5.16	5.41	94	12.4
27	0.003	0.027	1.81	0.42	67	72.9
29	0.001	0.028	1.08	0.35	95	96.5

[a] Concentration required for 50% inhibition of dilute prothrombin time.
[b] Concentration required to double plasma clotting time of APTT and PT, respectively.
[c] Percent compound remaining after 2 h incubation with human S9 liver fraction.
[d] Apparent permeability coefficient in Caco-2 cells.

activity in the standard coagulation assay for activated partial thromboplastin time (APTT) and prothrombin time (PT), as well as the more sensitive dilute PT (dPT) with diluted thromboplastin reagent.[10] Surprisingly, compounds **27** and **29**, which in the enzyme assay are approximately equipotent to **22** are around 16 times more potent than this compound in the more stringent and predictive dPT antithrombotic assay, as well as being more potent in the APTT and PT assays (Table 5).[10] This lack of clear correlation between enzyme inhibition and potency in coagulation assays has previously been reported.[5]

The same compounds were also tested in human S9 liver fractions and Caco-2 cell monolayers as in vitro predictive assays for metabolism and permeation, respectively. In these assays (Table 5) the compounds proved to be metabolically stable and, with the exception of **17**, showed excellent predicted absorption properties.[11]

In conclusion, we have developed a new class of potent nonguanidino/amidino fXa inhibitors based on a simple 2-carboxyindole scaffold. We have investigated the structural requirements for neutral P1 ligands in combination with this scaffold, and used these to develop a series of selective, structurally diverse, equipotent inhibitors. The compounds are predicted to be metabolically stable and well absorbed. They are capable of reducing the dilute prothrombin time, a 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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References and notes

1. (a) Hirsh, J. *N. Eng. J. Med.* **1991**, *324*, 156; (b) Freedman, M. D. *Am. J. Ther.* **1996**, *3*, 771.
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