Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4197–4201
Bioorganic & Medicinal Chemistry Letters

Novel factor Xa inhibitors based on a 2-carboxyindole scaffold: SAR of P4 substituents in combination with a neutral P1 ligand

Marc Nazaré,* Melanie Essrich, David W. Will, Hans Matter, Kurt Ritter, Matthias Urmann, Armin Bauer, Herman Schreuder, Jörg Czech, Martin Lorenz, Volker Laux and Volkmar Wehner

Aventis Pharma Deutschland GmbH, D-65926 Frankfurt am Main, Germany

Received 27 April 2004; accepted 8 June 2004

Abstract—A series of novel, highly potent 2-carboxyindole-based factor Xa inhibitors is described. Structural requirements for P4 ligands in combination with a neutral biaryl P1 ligand were investigated with the 2-carboxyindole scaffold. A diverse set of P4 substituents was identified, which, in conjunction with a biaryl P1 ligand, gave highly potent factor Xa inhibitors, which were also selective versus other proteases and efficacious in various antithrombotic secondary assays.

1. Introduction

The serine protease factor Xa (fXa) has emerged as a key target for the control and prevention of thrombogenesis associated with diseases like myocardial infarction, stroke, deep vein thrombosis and pulmonary embolism.[1] Its unique position along the blood coagulation cascade linking the intrinsic and extrinsic activation pathways combined with its role in thrombin activation has led to the expectation that inhibitors of fXa should be highly effective antithrombotics with fewer side effects and difficulties requiring monitoring of drug levels, slow onset of action, mode of administration and potentially life-threatening bleeding encountered with current available treatment.[2] Among the plethora of highly active selective fXa inhibitors discovered many share an amidine or guanidine moiety designed to bind in the S1 pocket of the enzyme. In general the presence of these highly basic groups results in poor oral absorption and have hindered the development of an orally bioavailable drug.[3] Three main strategies have been exploited to meet this challenge: the first is to mask the amidine as a prodrug;[4] the second is to reduce the basicity of the amidine by replacing it with various amidine mimics addressing the same polar interaction points at the cost of lower affinity.[5]

The third is to seek new types of interaction, such as the chloro binding mode, recently characterized by X-ray cocrystallization,[6] by incorporating a neutral chloroaryl P1 ligand. In the accompanying communication[7] we described the optimization of the neutral P1 ligand of fXa inhibitors based on a 2-carboxyindole scaffold. We succeeded in generating a set of equipotent inhibitors with structurally diverse neutral P1 substituents. These inhibitors were also shown to have improved Caco-2 permeability compared to amidine/guanidine compounds. We next turned our attention to the P4 ligand of fXa inhibitors based on the 2-carboxyindole scaffold. Interestingly, the S4 pocket of fXa is more conformationally flexible than the S1 pocket and thus P4 ligands with the correct orientation may bind by induced fit. We intended therefore to exploit this potential for structural flexibility by mapping the SAR of the P4 ligand in combination with a neutral P1 group interacting with the rigid S1 pocket of fXa.

In addition, we[8] and others[9] have observed that the potency of fXa inhibitors in more clinically-relevant assays of antithrombotic activity, such as activated partial thromboplastin time (APTT), prothrombin time (PT) and dilute PT (dPT), is not solely determined by the K_i value of the inhibitor on isolated fXa enzyme, but

Keyword: Factor Xa inhibitors.
* Corresponding author. Tel.: +49-69-305-15150; fax: +49-69-331-399; e-mail: marc.nazare@aventis.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.06.019

is driven by a complex interplay of several parameters, such as protein binding and polarity. In order to be able to tune these parameters we sought to generate a set of interchangeable P4 substituents to be combined with the P1 ligands we reported previously.[7]

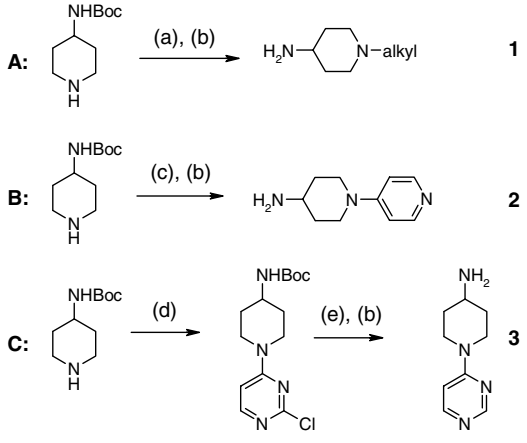
2. Chemistry

Scheme 1 illustrates the representative synthesis of prototypic building blocks **1**, **2**, **3** serving as putative P4 substituents. The N-alkyl derivatives **1** were obtained by subjecting commercially available N-Boc protected 4-aminopiperidine to standard reductive amination or alkylation procedures followed by acid catalyzed Boc deprotection. The corresponding 4-pyridyl compound **2** was prepared by *ipso* substitution of 4-chloropyridine and subsequent cleavage of the Boc-protecting group. A similar sequence extended by a catalytic dehalogenation led to pyrimidine **3**.

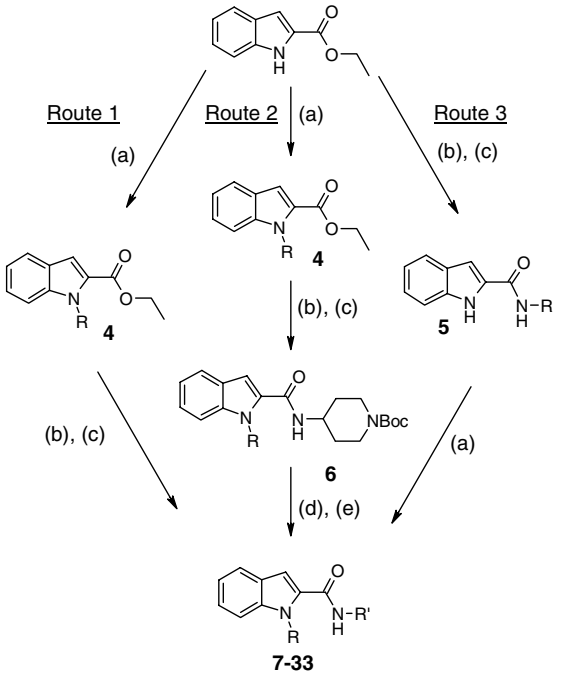
Scheme 2 shows the synthetic routes to the inhibitors described. Pre-assembly of the P4 ligands (**1**–**3**, and their analogues) followed by coupling to the 2-carboxyindole scaffold, which could be pre-derivatized (route 1) or post-derivatized (route 2) with the biaryl P1 ligand, led to the derivatives **7**–**33**. Alternatively, Boc-protected 4-aminopiperidine could be attached to the scaffold to give compound **6**. Following deprotection of the Boc group the terminal substituent could be introduced by, for example, reductive amination (route 3). Other P4 ligands were constructed and introduced analogously by one of these routes.

3. Results and discussion

Figure 1 shows the strategy employed to probe the SAR of the P4 ligand. The P1 group and the carboxyindole



Scheme 1. Reagents and conditions: (a) aldehyde or ketone $Na(CN)BH_3$, acetic acid, methanol, rt, 16 h or alkyl halide, NEt_3, DMF, rt, 4–16 h; (b) HCl, methanol, rt, 16 h; (c) 4-chloropyridine, *n*-butanol/water/NEt_3 1:1:1, 100 °C, 48 h; (d) 2,4-dichloropyrimidine, *n*-butanol/water/NEt_3 1:1:1, 100 °C, 48 h; (e) H_2, Pd/C, ethanol, acetic acid, rt, 5 h.



Scheme 2. Reagents and conditions: (a) R–Br, NaH, DMF, 80 °C, 1 h; (b) LiOH, THF/H_2O, 60 °C, 2 h; (c) amine, TOTU, N-ethylmorpholine, CH_2Cl_2, or BOP–Cl, NEt_3, CH_2Cl_2; (d) HCl, methanol, rt, 2 h; (e) aldehyde or ketone $Na(CN)BH_3$, acetonitrile, rt, 16 h or alkyl halide, NEt_3, DMF, rt, 4–16 h.

scaffold were kept constant throughout. The biaryl P1 ligand, which binds in the S1 pocket via the chloro binding mode driven by water displacement, was previously found to be one of the most potent in a series of related ligands.[7] Initially the 4-aminopiperidine 'spacer' was held constant, and simple alkyl terminal substituents were introduced. Subsequently, more polar terminal groups were introduced onto the 4-aminopiperidine moiety, as well as heterocycles, which had previously been found to be active in combination with other scaffolds.[6] Ring-contracted analogues of the 4-aminopiperidine moiety were introduced, both as simple alkyl derivatives and derivatized with heterocycles. Finally, a variety of other related P4 ligands with, for example, reversed, extended or contracted spacers were investigated.

Table 1 summarizes the results for fXa inhibitors **7**–**15** with P4 substituents composed of a 4-aminopiperidine 'spacer' and simple alkyl/cycloalkyl terminal substituents. Compounds **7**–**9** show that of the simple unbranched alkyl termini, ethyl has the optimal size. Compound **10**, with an isopropyl terminus, the smallest possible branched alkyl substituent, is the most potent compound in this series. Increasing the size of the hydrophobic terminus, in compounds **11** and **12**, as well as in the cycloalkyl derivatives **14** and **15** was also deleterious to potency. Surprisingly, cyclopropyl-substituted compound **13** is nine times less potent than the isopropyl derivative **10**, although both compounds are essentially isosteric. This can be explained by the reduction in basicity of the cyclopropyl-substituted piperidine nitrogen compared to its isopropyl counter-

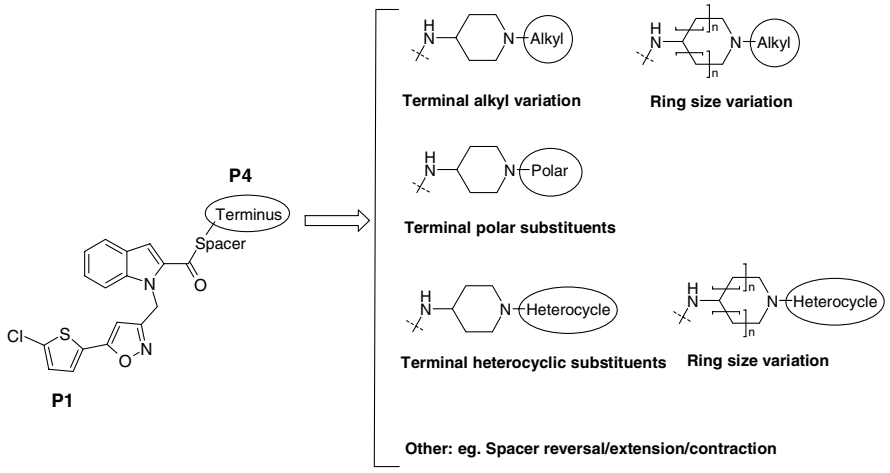
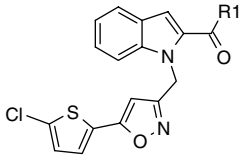
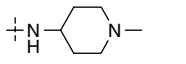
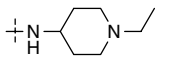
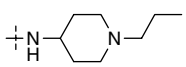
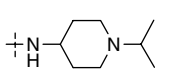
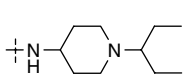
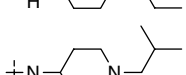
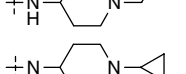
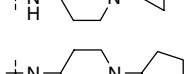
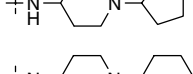


Figure 1. Schematic diagram summarising the P4 ligand modification strategy.

part. The measured pK_a value for compound **13** was 6.5, compared to 8.6 for compound **10**, due to the vinylic character of the cyclopropyl group.[11] This clearly demonstrates that it is not only hydrophobic interactions, which are important for potency in the S4 pocket, but also the piperidine nitrogen is involved in polar interactions and plays a critical role to fill the S4 cation hole. Compound **16** in Table 2 is the 'reversed linker' analogue of compound **10**, and is 1000-fold less potent. A considerable, but less dramatic drop in activity is observed on extension of the linker by one methylene unit (**17**). Compounds **18** and **19**, which retain the tertiary amine of the piperidine linker, but have a hydrophobic terminus have similar activity to those compounds in Table 1 with larger purely hydrophobic termini. Acetyl, methylsulfonyl and urea derivatives **20**–**22**, which were designed to be highly polar, but approximately isomorphous with the isopropyl group, are also considerably less active than **10**, again confirming the preference for a basic nitrogen in the P4 ligand.

Table 1. 2-Carboxyindole-based factor Xa inhibitors: SAR of variation of the alkyl substituent on 4-aminopiperidine P4 ligands



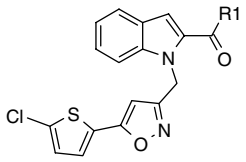
Compds	R1	K_i (fXa) nM
7		40
8		18
9		64
10		3
11		220
12		142
13		27
14		71
15		106

Ring-contraction gave aminopyrrolidine derivatives (**23** and **24**) of reasonable potency, whereby one enantiomer was twice as active as the other. Further ring-contraction to give aminoazetidine derivative **25** resulted in a somewhat more significant drop in activity. Compounds **26** and **27** were synthesized in an attempt to compensate for the shortening of the spacer compared to the original aminopiperidine, however they brought no improvement in activity.

The results in Tables 1 and 2 demonstrate that a relatively broad range of terminal substituents is tolerated in the S4 pocket, with very few modifications resulting in a dramatic loss in potency. This is indicative of the conformational flexibility of the S4 pocket of fXa.

In our previous work on fXa inhibitors based on a benzoic acid scaffold[8] we found that a 4-aminopyridine terminus to the P4 ligand was optimal for that scaffold. In spite of the results shown in Tables 1 and 2, which would suggest that a pyridyl terminus to the P4 ligand would be too large to be highly active, we synthesized the series of compounds shown in Table 3. Remarkably, compound **28**, which incorporates the 4-aminopiperidine spacer with a 4-pyridyl terminal substituent, has a K_i value of 14 nM. Replacement of the 4-aminopyridyl motif by the corresponding pyrimidine (**29**) resulted in a sevenfold loss in potency. Extension of the linker by one methylene unit (**30**) also resulted in a drop in activity, but the effect was not as pronounced as that caused by

Table 2. 2-Carboxyindole-based factor Xa inhibitors: SAR of non-aromatic P4 ligands



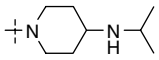
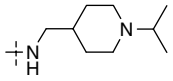
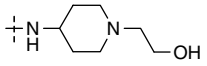
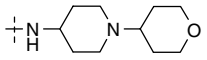
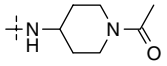
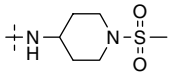
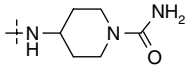
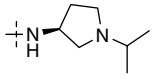
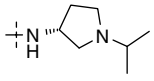
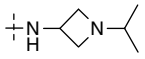
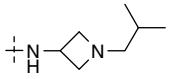
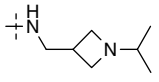
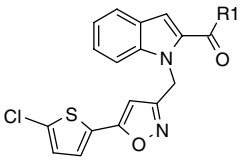
Compds	R1	K_i (fXa) nM
16		2997
17		161
18		131
19		95
20		763
21		125
22		1075
23		103
24		48
25		225
26		345
27		839

Table 3. 2-Carboxyindole-based factor Xa inhibitors: SAR of aromatic P4 ligands



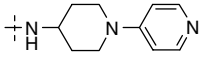
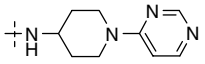
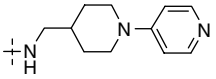
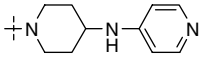
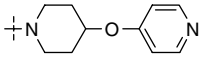
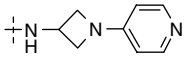
Compds	R1	K_i (fXa) nM
28		14
29		95
30		64
31		18
32		2900
33		12

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
7	0.040	>10	>100	>10	>100
8	0.018	>10	>100	>10	>100
10	0.003	2.76	>100	>10	>100
13	0.027	8.22	>100	>10	>100
24	0.048	>10	>100	>10	>100
28	0.014	>10	>100	>10	>100
31	0.018	3.163	>100	8.54	64.01
33	0.012	>10	>100	>10	>100

[a] Human tissue plasminogen activator.

Table 5. Anticoagulant activity of selected compounds

Compds	fXa K_i/μM	dPT[a] μM	APTT[b] μM	PT[b] μM
7	0.040	1.554	40.65	30.69
8	0.018	0.377	13.79	9.02
9	0.064	1.563	38.18	15.58
10	0.003	0.426	5.16	5.41
13	0.027	1.799	27.53	19.58
24	0.048	0.625	16.48	13.72
28	0.014	0.232	8.260	6.04
31	0.018	0.198	11.63	5.78
33	0.012	0.379	4.380	6.20

[a] Concentration required for 50% inhibition of dilute prothrombin time (dPT).[10]

[b] Concentration required to double plasma clotting time of APTT and PT, respectively.

the same modification in the alkyl series (cf. **10** and **17**). Even more remarkably, compound **31**, in which the 4-aminopiperidine spacer is reversed, is approximately equipotent to **28**. This is in stark contrast to the dramatic 1000-fold drop in affinity caused by the same modification in the alkyl series described above (cf. **10** and **16**). This result also demonstrates that there is no essential NH-interaction of the carboxyindole amide with the enzyme. The critical role played by the 4-aminopyridyl motif in this series, can be seen clearly by comparison of compounds **31** and **32** where replacement of the amino nitrogen by an oxygen results in a more than 160-fold drop in affinity. Ring-contraction of the spacer

to an azetidine (**33**) in combination with the 4-pyridyl terminus resulted in an inhibitor equipotent to **28**.

The surprising results shown in Table 3 tend to confirm the conformational flexibility of the S4 pocket and suggest that the P4 ligands may indeed bind by induced fit.

Key compounds were further characterized against related proteases and display a high degree of selectivity (Table 4). This favourable selectivity pattern can be mainly attributed to the biarylic P1 substituent employing a chloro binding mode and the other 2-carboxyindole structural determinants common to all inhibitors.

Table 5 shows the evaluation of the anticoagulant activity in the standard coagulation assays for activated partial thromboplastin time (APTT) and prothrombin time (PT), as well as the more sensitive dilute PT (dPT) with diluted thromboplastin reagent.[10] Again this confirmed our previous observation that there is no clear correlation between the K_i value on the isolated enzyme and the clinically-relevant antithrombotic surrogate parameters. For example, compound **10** is no more active than compounds **8**, **28**, **31**, **33** in the dPT assay in spite of being four to six times more potent on fXa.

In summary, we have synthesized a series of 2-carboxyindole inhibitors with a neutral biarylic P1 group and cyclic amine-derived P4 ligands and demonstrated that they are potent and selective inhibitors of fXa. In this context structural requirements in the S4 pocket of the enzyme were revealed and exploited to generate a set of structural diverse inhibitors. Several compounds were identified, which were capable of reducing the dilute prothrombin time, a recognized parameter for in vivo antithrombotic activity in the clinic. Hence, a new generation of potent and selective fXa inhibitors with a broad variation in the P4 region and with a neutral P1 group emerged, which are promising drug substances for the therapeutic prevention and control of thrombotic diseases.

Acknowledgements

We would like to thank Astrid Sihorsch, Marga Kämmerer-Dienst, Lynn Bayer, Dirk Timme, Norbert Laub and Stefan Engel for their excellent technical assistance.

References and notes

1. Leadley, R. J. *Curr. Top. Med. Chem.* **2001**, *1*, 151.
2. Kaiser, B. *Drugs Future* **1998**, *23*, 423.
3. Pauls, H. W.; Ewing, W. R. *Curr. Top. Med. Chem.* **2001**, *1*, 83.
4. (a) Song, Y.; Clizbe, L.; Bhakta, C.; Teng, W.; Wong, P.; Huang, B.; Tran, K.; Sinha, U.; Park, G.; Reed, A.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 297; (b) Gustafsson, D.; Elg, M. *Thromb. Res.* **2003**, *109*, S9.
5. (a) Pinto, D. J. P.; Orwat, M. J.; Wang, S.; Fevig, J. M.; Quan, M. L.; Amparo, E.; Cacciola, J.; Rossi, K. A.; Alexander, R. S.; Smallwood, A. M.; Luettgen, J. M.; Liang, L.; Aungst, B. J.; Wright, M. R.; Knabb, R. M.; Wong, P. C.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2001**, *44*, 566; (b) Lam, P. Y. S.; Clark, C. G.; Li, R.; Pinto, D. J. P.; Orwat, M. J.; Galemmo, R. A.; Fevig, J. M.; Teleha, C. A.; Alexander, R. S.; Smallwood, A. M.; Rossi, K. A.; Wright, M. R.; Bai, S. A.; He, K.; Luettgen, J. M.; Wong, P. C.; Knabb, R. M.; Wexler, R. R. *J. Med. Chem.* **2003**, *46*, 4405.
6. (a) Adler, M.; Kochanny, M. J.; Ye, B.; Rumennik, G.; Light, D. R.; Biancalana, S.; Whitlow, M. *Biochemistry* **2002**, *41*, 15514; (b) Maignan, S.; Guilloteau, J.-P.; Choi-Sledeski, Y. M.; Becker, M. R.; Ewing, W. R.; Pauls, H. W.; Spada, A. P.; Mikol, V. *J. Med. Chem.* **2003**, *46*, 685.
7. Nazaré, M.; Essrich, M.; Will, D. W.; Matter, H.; Ritter, K.; Urmann, M.; Bauer, A.; Schreuder, H.; Dudda, A.; Czech, J.; Lorenz, M.; Laux, V.; Wehner, V. *Bioorg. Med. Chem. Lett.* **2004**, *14*, preceding paper in this issue. doi:10.1016/j.bmcl.2004.06.020.
8. Nazaré, M.; Matter, H.; Klingler, O.; Al-Obeidi, F.; Schreuder, H.; Zoller, G.; Czech, J.; Lorenz, M.; Dudda, A.; Peyman, A.; Nestler, H. P.; Urmann, M.; Bauer, A.; Laux, V.; Wehner, V.; Will, D. W. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2801.
9. Jia, Z. J.; Wu, Y.; Huang, W.; Zhang, P.; Clizbe, L. A.; Goldman, E. A.; Sinha, U.; Arfsten, A. E.; Edwards, S. T.; Alphonso, M.; Hutchaleelaha, A.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1221.
10. In vitro assays for determination of fXa and thrombin activity were performed as described by Ostrem, J. A.; Al-Obeidi, F.; Safar, P.; Safarova, A.; Stringer, S. K.; Patek, M.; Cross, M. T.; Spoonamore, J.; LoCascio, J. C.; Kasireddy, P.; Thorpe, D. S.; Sepetov, N.; Lebl, M.; Wildgoose, P.; Strop, P. *Biochemistry* **1998**, *37*, 1053.
11. (a) pK_a values were measured by UV spectroscopy on a Sirius SGA profiler; (b) Perrin, C. L.; Fabian, M. A.; Rivero, I. A. *Tetrahedron* **1999**, *55*, 5773; (c) Gillaspy, M.; Lefker, B. A.; Hada, W. A.; Hoover, D. J. *Tetrahedron Lett.* **1995**, *36*, 7399.