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Solid-Phase Optimisation of Achiral Amidinobenzyl Indoles as Potent and Selective Factor Xa Inhibitors

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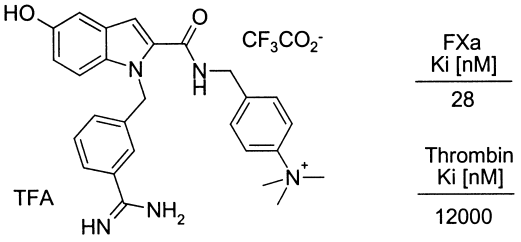
Abstract—Starting from the achiral and potent factor Xa inhibitor **1**, a new and flexible solid-phase optimisation strategy is described to reduce its cationic character. By replacing one positively charged side chain by a lipophilic substituent, a novel series of highly potent and selective achiral factor Xa inhibitors was discovered. The identified lipophilic replacements in the S4 pocket might be valuable for other approaches towards fXa inhibitors. © 2001 Elsevier Science Ltd. All rights reserved.

The blood coagulation serine protease factor Xa (fXa) plays a crucial role in the coagulation pathway by activating prothrombin to generate thrombin. Thrombin converts fibrinogen to fibrin for clot formation and induces platelet aggregation. Both incidents lead to serious pathological situations. The inhibition of fXa compared to thrombin inhibition may allow the effective control of thrombogenesis with a minimal effect upon bleeding.[1–3]



FXa Ki [nM]: 28

Thrombin Ki [nM]: 12000

1

The growing interest in fXa inhibitors is reflected by the increasing number of publications in this field.[4–13] Several pharmaceutical companies are currently searching for intravenously and especially orally active fXa inhibitors.[4] During our own work the hydroxyindole-2-carboxamide **1** was identified as a potent and selective fXa inhibitor.[14] The advantage of this compound compared to many of the known fXa inhibitors is the simplicity of its structure (no chiral centre, no *E*/*Z* double bond isomers) and as a result thereof, the ease of its synthesis (building blocks are easily synthesised by standard methods or commercially available, no isomer separation).[4–13]

Although both cationic sites (amidine and ammonium) are clearly favourable for affinity they seemed to be detrimental to oral bioavailability and other pharmacokinetic properties. Therefore, a solid-phase optimisation strategy was envisaged to reduce the cationic character of **1**. The flexibility of this approach is shown in Figure 1. R1, R2 and X are variable allowing a fully combinatorial approach. This indole core structure represents an interesting scaffold giving access to a variety of different library approaches.

In this first investigation we concentrated on variations at R1 and X keeping R2 constant, since first derivatives from single compound synthesis suggested that the *m*-benzamidine substituent at R2 is highly favourable for affinity in this series.

Design

After validation of the solid-phase reaction, adequate building blocks were extracted from ACD[15] using substructure searching, filtered by a list of preferred suppliers

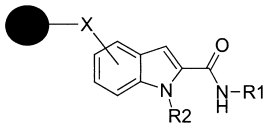


Figure 1.

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and appropriate physicochemical property thresholds.[16] Reactants with multiple occurrences of the reactive functionality or undesirable functionalities were rejected.[17] An optimised set of building blocks was obtained by flexibly docking a virtual library of all possible products into the fXa ligand binding site and optimising the binding geometry by energy minimisation.[18,19] In general the *m*-substituted benzamidine was proposed to fit into the S1 pocket and considered essential for affinity towards fXa in this series. In order to reduce the overall basic character of the proposed inhibitors, mainly hydrophobic substituents directed towards the S4 pocket were included. All final protein–ligand complexes were subsequently scored using a 3D-QSAR model (CoMFA)[20] built from a previous set of indole fXa inhibitors. Acceptable commercially available building blocks were utilised for subsequent solid-phase synthesis.

Synthesis

The reaction sequence is shown in Scheme 1.[21] The amino- or hydroxyindole ester **2** was coupled to a polystyrene resin with a 2-chlorotritylchloride linker **3**. The coupled indole **4** was *N*-alkylated with 3-cyano-benzyl bromide (**5**) in the presence of the strong base 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) (**6**).[22] The replacement of the diazaphosphorine by the weaker base *N*,*N*-diisopropylethylamine (DIEA) did not lead to any alkylation product. Saponification of indole ester **7** by lithium hydroxide in a mixture of THF and water (70:30) was not successful. In contrast, benzyltrimethylammonium hydroxide in a mixture of methanol (MeOH) and *N*,*N*-dimethylformamide (DMF) gave a clean conversion to the acid, that was coupled with the respective amine in the presence of *N*,*N*′-diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole hydrate (HOBt) to yield the amide **8**. Using H_2S, pyridine (py), and triethylamine (TEA) the nitrile functionality was converted to the thioamide that was treated with methyl iodide (MeI) in acetone followed by ammonium acetate in a mixture of MeOH and acetic acid to yield the amidine **9**.[23] The resin bound compound was cleaved by a mixture of dichloromethane (DCM), trifluoro acetic acid (TFA), and water to give the final product **10**. The purity of the crude material was determined by HPLC. Pure compounds were obtained by preparative HPLC purification. The products were characterised by HPLC, MS, and ^{1}H NMR.

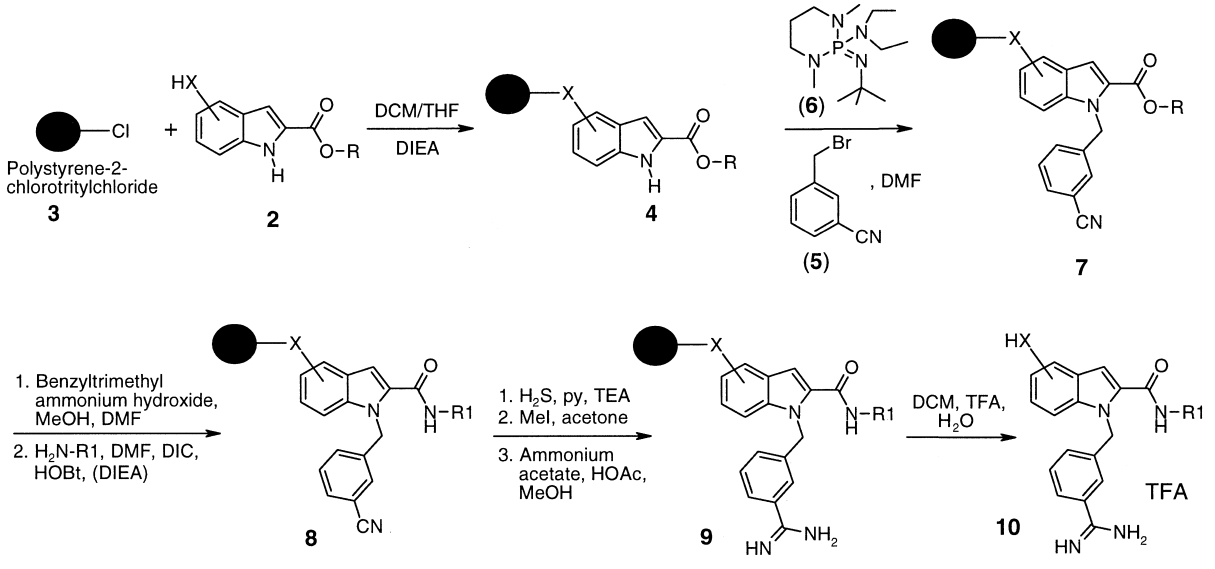
So far about 50 compounds have been synthesised by means of parallel synthesis in syringes. The HPLC purity of most of the crude products was in the range of 65–85% detected at 254 nm. After preparative HPLC purification the amount of purified product was in the range of 5–25 mg.

Results and Discussion

All compounds were assayed for their inhibitory activity against fXa.[24] In many cases the purity of the crude material was sufficient for biological testing. For selected compounds their activity against thrombin was also determined.[24] The results for a representative set of compounds are shown in Table 1.

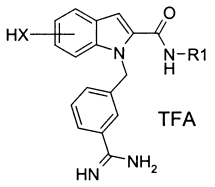
Two compounds, **10f** and **10h**, show very good affinity to fXa with K_i values of 5 and 11 nM, respectively. For **10f** the selectivity towards thrombin is greater than two orders of magnitude.

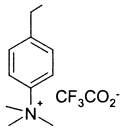
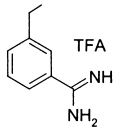
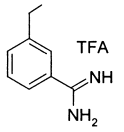
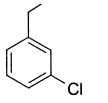
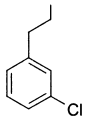
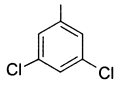
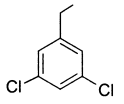
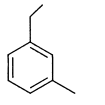
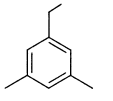
The most active indoles identified so far have a hydroxy group in the 4-position. As best trimethylphenylammonium replacements, 3,5-disubstituted phenyl derivatives were discovered, possessing one methylene bridge between the amide nitrogen and the phenyl ring (**10f** and **10h**). If the phenyl ring is either directly linked to the nitrogen (**10e**) or via an ethylene group (**10d**) the activity against fXa is reduced. The presence of the second substituent in the 5-position of the side-chain ring (R1) increased the activity against fXa (**10c** versus **10f**, **10g** versus **10h**).



Scheme 1.

Table 1.



Compound	X	R1	FXa K_i (nM)	Thr K_i (nM)
1	5-O		28	12,000
10a	5-NH×TFA		84	nD
10b	4-O		9	3600
10c	4-O		262	nD
10d	4-O		707	nD
10e	4-O		798	nD
10f	4-O		5	771
10g	4-O		131	nD
10h	4-O		11	620

The discovery of 3,5-dichloro and 3,5-dimethylphenyl as replacements for trimethylphenylammonium or benzamidine is an important step towards orally available fXa inhibitors within this indole series. They might also represent valuable building blocks in the S4 position for other approaches towards fXa inhibitors.[4–13] Interestingly, chlorophenyl derivatives were also identified as potent lipophilic replacements for cationic groups in the S1 pocket of thrombin.[25]

In conclusion, the achiral indoles **10f** and **10h** show nanomolar activity against fXa and maintain high selectivity over thrombin. The positive character in these compounds compared to **1**, **10a** and **10b** was reduced by replacing the trimethylphenylammonium or benzamidine moiety against 3,5-dichloro and 3,5-dimethylphenyl moieties. The compounds were synthesised via a newly developed solid-phase optimisation strategy allowing variations at three different sites.

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References and Notes

1. Harker, L. A.; Hanson, S. R.; Kelly, A. B. *Thromb. Haemostasis* **1995**, *74*, 464.
2. Waxman, L.; Smith, D. E.; Arcuri, K. E.; Vlasuk, G. P. *Science* **1990**, *248*, 593.
3. Hara, T.; Yokoyoma, A.; Tanabe, K.; Ishihara, H.; Iwamoto, M. *Thromb. Haemostasis* **1995**, *74*, 635.
4. Al-Obeidi, F.; Ostrem, J. A. *Exp. Opin. Ther. Patents* **1999**, *9*, 931.
5. Fevig, J. M.; Buriak, J., Jr.; Stouten, P. F. W.; Knabb, R. M.; Lam, G. N.; Wong, P. C.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1195.
6. Guilford, W. J.; Shaw, K. J.; Dallas, J. L.; Koovakkat, S.; Lee, W.; Liang, A.; Light, D. R.; McCarrick, M. A.; Whitlow, M.; Ye, B.; Morrissey, M. M. *J. Med. Chem.* **1999**, *42*, 5415.
7. Quan, M. L.; Liauw, A. Y.; Ellis, C. D.; Pruitt, J. R.; Carini, D. J.; Bostrom, L. L.; Huang, P. P.; Harrison, K.; Knabb, R. M.; Thoolen, M. J.; Wong, P. C.; Wexler, R. R. *J. Med. Chem.* **1999**, *42*, 2752.
8. Quan, M. L.; Ellis, C. D.; Liauw, A. Y.; Alexander, R. S.; Knabb, R. M.; Lam, G. N.; Wright, M. R.; Wong, P. C.; Wexler, R. R. *J. Med. Chem.* **1999**, *42*, 2760.
9. Ewing, W. R.; Becker, M. R.; Manetta, V. E.; Davis, R. S.; Pauls, H. W.; Mason, H.; Choi-Sledeski, Y. M.; Green, D.; Cha, D.; Spada, A. P.; Cheney, D. L.; Mason, J. S.; Maignan, S.; Guilloteau, J.-P.; Brown, K.; Colussi, D.; Bentley, R.; Bostwick, J.; Kasiewski, C. J.; Morgan, S. R.; Leadley, R. J.; Dunwiddie, C. T.; Perrone, M. H.; Chu, V. *J. Med. Chem.* **1999**, *42*, 3557.
10. Kucznierz, R.; Grams, F.; Leinert, H.; Marzenell, K.; Engh, R. A.; von der Saal, W. *J. Med. Chem.* **1998**, *41*, 4983.
11. Renatus, M.; Bode, W.; Huber, R.; Stürzebecher, J.; Stubbs, M. T. *J. Med. Chem.* **1998**, *41*, 5445.
12. Marlowe, C. K.; Uma, S.; Gunn, A. C.; Scarborough, R. M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 13.
13. Gong, Y.; Pauls, H. W.; Spada, A. P.; Czekaj, M.; Liang, G.; Chu, V.; Colussi, D. J.; Brown, K. D.; Gao, J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 217.
14. Defossa, E.; Heinelt, U.; Klingler, O.; Zoller, G.; Al-Obeidi, F.; Walser, A.; Wildgoose, P.; Matter, H. PCT Int. Appl. 1999, 199 pp WO 9933800 A1 19990708.
15. ACD: Available Chemical Directory, from Molecular Design Ltd; 14600 Catalina Street, San Leandro, CA 94577.
16. Ferguson, A. M.; Patterson, D. E.; Garr, C.; Underiner, T. *J. Biomol. Screen* **1996**, *1*, 65.
17. Rishton, G. M. *Drug. Disc. Today* **1997**, *2*, 382.

18. Padmanabhan, K.; Padmanabhan, K. P.; Tulinsky, A.; Park, C. H.; Bode, W.; Huber, R.; Blankenship, D. T.; Cardin, A. D.; Kisiel, W. *J. Mol. Biol*. **1993**, *232*, 947.
19. All modelling work was done using Sybyl 6.5 (SYBYL, Version 6.5, Tripos, St. Louis, MO, 1999). Flexible docking was done using a genetic algorithm as implemented in Flexi-Dock. Energy calculations were based on the TRIPOS 6.0 force field (Clark, M.; Cramer, R. D., III, Van Opdenbosch, N. *J. Comp. Chem*. **1989**, *10*, 982) with Gasteiger–Marsili charges (Gasteiger, J.; Marsili, M. *Tetrahedron* **1980**, *36*, 3219). Protein–ligand complexes were minimised using the quasi-Newton–Raphson (BFGS) procedure (500 steps).
20. Cramer, R. D.; Patterson, D. E.; Bunce, J. E. *J. Am. Chem. Soc*. **1988**, *110*, 5959.
21. Synthesis of **10b** (a general protocol, the reaction steps were monitored by cleaving a small sample of resin after each step and HPLC-MS analysis): **4b**: 4-Hydroxy-1*H*-indole-2-carboxylic acid ethyl ester (345 mg, 1.69 mmol) was dissolved in 11 mL of a DCM/THF mixture (10:1). DIEA (487 μL, 2.8 mmol) was added and the mixture was sucked into a syringe fitted with a polyethylene sheet and containing the 2-chlorotritylchloride polystyrene resin **3** (535 mg, 0.56 mmol; substitution 1.05 mmol/g, Novabiochem). After shaking for 2 h at room temperature (rt) the mixture was removed and the resin was washed with DCM. A mixture of MeOH (91 μL), DIEA (389 μL), and DCM (10 mL) was added and the syringe was shaken at rt. After 1.5 h the mixture was removed, the resin was washed with DMF, DCM, and MeOH and dried in vacuo. **7b**: **4b** was shaken in DMF for 5 min, washed with DMF and then a mixture of BEMP (**6**) (405 μL, 1.4 mmol) and DMF (5 mL) was added to the resin. After shaking for 1 h 3-cyano-benzyl bromide (**5**) (220 mg, 1.12 mmol) dissolved in DMF (5 mL) was added. The mixture was removed 3 h later, the resin was washed with DMF and MeOH and dried in vacuo. **8b**: **7b** was shaken for 5 min with DMF (5 mL). After the removal of the DMF a mixture of benzyltrimethylammonium hydroxide (40% in MeOH; 5.6 mmol, 2.5 mL) and DMF (15 mL) was sucked in and shaken for ~5 h. After removal of the mixture the resin was washed with DMF and MeOH and dried in vacuo. A portion of this resin (98 of 512 mg, ~0.1 mmol) was shaken for 5 min with DMF before the reagent mixture consisting of 3-aminomethylbenzonitrile hydrobromide (64 mg, 0.3 mmol), DIEA (70 μL, 0.4 mmol), DIC (44 mg, 0.35 mmol), and HOBt hydrate (54 mg, 0.4 mmol) in DMF (4 mL) was added. After 16 h the reaction mixture was removed and the resin was washed with DMF, MeOH, and DCM and dried in vacuo. **9b**: **8b** was shaken in 2 mL py/TEA (2:1) for 15 min. The solution was removed, a saturated solution of H_2S in py/TEA (2:1) (1 mL) was added and the mixture was shaken overnight. Then the H_2S solution was removed and the resin was washed with acetone. The acetone was replaced by a solution of MeI (250 μL) in acetone (2 mL) and the syringe was shaken overnight. Then the MeI solution was removed and the resin washed with acetone and MeOH. A solution of ammonium acetate (210 mg), acetic acid (100 μL), and MeOH (2 mL) was added and the syringe heated at 50 °C for 3 h. Then the solution was removed and the resin washed with MeOH, DMF, and DCM. **10b**: **9b** was treated with a mixture of DCM, TFA, and water (60:40:0.1). After 15 min the cleavage mixture was transferred into a flask and the resin was washed with MeOH. The MeOH washes were added to the cleavage mixture and the resulting solution was evaporated in vacuo. The residue was dissolved in water/acetonitrile and purified by preparative HPLC on a C_{18} column using a water/acetonitrile gradient. Pure fractions were combined and after lyophilisation 11 mg (~16%) of **10b** (white solid) was obtained. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.89 (s, 1), 9.32 (br s, 4), 9.17 (t, 1), 9.09 (br s, 4), 7.77–7.75 (m, 2), 7.70–7.55 (m, 4), 7.46 (m, 1), 7.40 (s, 1), 7.17–7.14 (m, 1), 7.02 (t, 1), 6.88–6.86 (m, 1), 6.48–6.45 (m, 1), 5.87 (s, 2), 4.54 (d, 2), MS 441.0 ($M + H^+$).
22. Schwesinger, R.; Willaredt, J.; Schlemper, H.; Keller, M.; Schmitt, D.; Fritz, H. *Chem. Ber*. **1994**, *127*, 2435.
23. Wagner, G.; Voigt, B.; Vieweg, H. *Pharmazie* **1984**, *39*, 226.
24. 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.
25. Tucker, T. J.; Brady, S. F.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Naylor-Olsen, A. M.; Yan, Y.; Sisko, J. T.; Stauffer, K. J.; Lucas, B. J.; Lynch, J. J.; Cook, J. J.; Stranieri, M. T.; Holahan, M. A.; Lyle, E. A.; Baskin, E. P.; Chen, I-W.; Dancheck, K. B.; Krueger, J. A.; Cooper, C. M.; Vacca, J. P. *J. Med. Chem*. **1998**, *41*, 3210.