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Inhibitors of the Tissue Factor/Factor VIIa-Induced Coagulation: Synthesis and In Vitro Evaluation of Novel Specific 2-Aryl Substituted 4*H*-3,1-Benzoxazin-4-ones

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Abstract—The synthesis of a series of novel 2-aryl substituted 4*H*-3,1-benzoxazin-4-ones and their evaluation as specific inhibitors of the Tissue Factor (TF)/Factor VIIa (FVIIa)-induced pathway of coagulation is reported. Inhibitory activities (IC_{50} values) in the range 0.17 to $>40\,\mu M$ on the activation of Factor X (FX) by the TF/FVIIa complex were found for compounds having one or two electronegative substituents such as F, Cl and NO_2 in the 2-aryl substituent. Different substitutions both electron-attracting and donating groups were allowed in the 5, 6, 7 and 8 positions. Several of the compounds showed a selectivity ratio towards FX and thrombin of >50, thus being the first small molecules described as potential drugs for oral antithrombotic treatment without side effects such as bleeding which is observed especially with thrombin inhibitors. The best substituent pattern being the 2-aryl group substituted with: 2-F; 2,6-F_2; or 2-FX; 6-Cl; together with electronegative substitution in the 5, 6, 7, or 8 positions. 2-Heteroaryl substituents like thienyl and furanyl were of low activity while some 2-(2-chloro-3-pyridyl) derivatives had inhibitory activity $<10\,\mu M$ and a good selectivity.

Introduction

Blood coagulation is a complex process involving various blood components or factors that eventually give rise to a fibrin clot. Activated Factor X (FXa) is required to convert prothrombin to thrombin which then converts fibrinogen to fibrin as a final stage in forming a fibrin clot.

There are two pathways that promote the activation of Factor X (FX). The 'intrinsic pathway' refers to those reactions leading to thrombin formation through utilisation of factors present only in blood. A series of protease-mediated activations generate Factor IXa which, in conjunction with Factor VIIIa, cleaves FX into FXa.

In the 'extrinsic pathway' Factor VIIa (FVIIa) and its cofactor, Tissue Factor (TF), exert the same proteolysis. TF is a membrane-bound protein and does not normally circulate in plasma. Upon vessel disruption, however, it is exposed and forms a complex with FVIIa to catalyse FX or Factor IX activation in the presence of Ca^{2+} and phospholipid (1). While the relative importance of the two coagulation pathways in hemostasis is unclear, FVIIa and TF appear to be the principal initiators of blood coagulation in vivo.[1]

It is often desirable to inhibit the coagulation cascade in a patient. Anticoagulants such as heparin, coumarin and its derivatives, indandione derivatives, low-molecular-weight thrombin or FXa inhibitors, or other agents may be used. Treatment with heparin and other anticoagulants may, however, have undesirable side effects, for example, bleedings. Inhibition at the initial stage of blood coagulation, i.e. of the FVIIa/TF activity, results in significantly less bleeding.[2] In addition, clinically available anticoagulants act throughout the body, rather than acting specifically at the site of injury where the coagulation cascade is active. Other known anticoagulants comprise thrombin and Factor Xa inhibitors derived from blood-sucking organisms. Antithrombins, hirudin, hirulog and hirugen are recombinant proteins or peptides derived from the leach *Hirudo medicinalis*, whereas the Factor Xa inhibitor antistatin and the recombinant derivative rTAP are tick-derived proteins. Inhibitors of platelet aggregation such as monoclonal antibodies or synthetic peptides, which interfere with the platelet receptor gpIIb/IIIa, are also effective as anticoagulants. These agents are, however, not suitable for oral administration. Specific protein inhibitors of the FVIIa/TF activity are the

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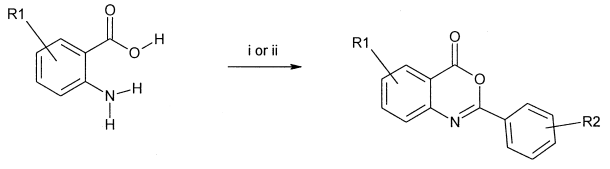
physiological inhibitor TFPI (Tissue Factor Pathway Inhibitor) and the inactivated FVII (FVIIai) which binds to TF and compete with FVIIa

There is still a need for improved compositions having anticoagulant activity and which can be administered orally or otherwise non-intravenously at relatively low doses without producing undesirable side effects. We found that some 2-arylsubstituted 4*H*-3,1-benzoxazin-4-ones showed specific inhibitory activity on FVIIa-TF in in vitro assays, and therefore seem to be potential drug candidates for blood coagulation regulation, suitable for oral administration and presumably without the above mentioned side effects. We report here the synthesis and SAR of some 2-arylsubstituted 4*H*-3,1-benzoxazin-4-ones in in vitro systems of TF/FVIIa-catalyzed activation of FX, as well as their effects on the amidolytic activity of FXa and thrombin and the prolongation of clotting time caused by selected compounds. 4*H*-3,1-Benzoxazin-4-one-derivatives are known to act as serine protease inhibitors, but very few of the reported compounds contain an aryl group connected directly to the ring system together with substituents in the 5, 6, 7, or 8 positions.[3–9] None of the previously described compounds have been reported to specifically inhibit the TF/FVIIa-induced coagulation pathway, but rather to act as thrombin and FXa inhibitors. Similar structures, often containing a 2-amino substituent instead of the 2-aryl substituent, are reported to act as inhibitors of HSV-1 protease,[10] C1r serine protease,[11] and human cytomegalovirus protease.[12] A solid-phase synthetic approach towards 2-amino substituted 4H-3,1-benzoxazin-4-ones has been reported.[13]

Chemistry

2-Aryl substituted 4*H*-3,1-benzoxazin-4-ones were conveniently prepared from optionally substituted anthranilic acids and an aryl carboxylic acid chloride in toluene or DMF using triethylamine or pyridine as base. However, the yields varied considerably (0% to 100%), depending on the substitution pattern in both the anthranilic acid and the aryl carboxylic acid or acid chloride. Syntheses using an aromatic *ortho* aminocarboxylic acid ester and an aryl carboxylic acid chloride as starting materials performing the ring closure with sulphuric acid[3] gave very low yields, and the same was found for syntheses using an anthranilic acid derivative and an arylcarboxylic acid performing the reaction with HOBT and EDAC, probably due to the presence of two carboxylic acids which could be activated by HOBT.[4–9] The reaction is depicted in Scheme 1.



Scheme 1. Synthetic pathway used for the preparation of compounds **2**, **5**, **17**–**20**, **22**, **24**, **27**, **28**, **30**, **32**, **33**, **36**, **38**, **44**, **59** and **60**. i: Ar(R2)-COCl, Et_3N, Toluene; ii: Ar(R2)COOH, HOBT, EDAC, DMF.

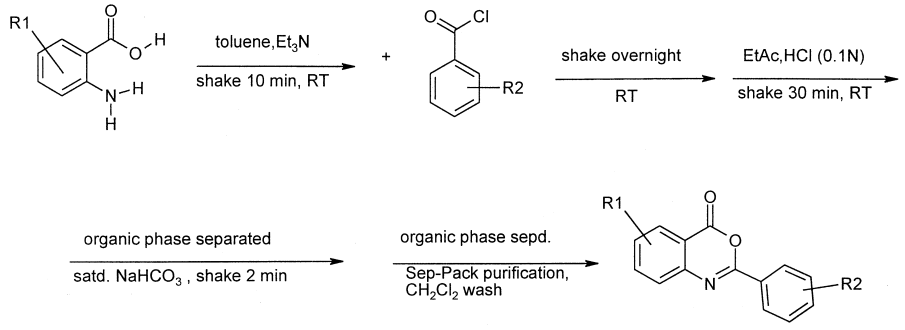
In order to conveniently prepare a series of analogues for the SAR investigation, a solution phase parallel synthesis procedure was developed (Scheme 2).

Appropriately substituted 2-aminobenzoic acids were dissolved in toluene, triethyl amine was added and the mixture shaken for 10 min at room temperature. Subsequently, substituted benzoic acid chloride was added and the mixture shaken overnight at room temperature. Ethyl acetate and HCl (0.1N) were added followed by shaking. The organic layer was separated, saturated $NaHCO_3$ added, and the mixture shaken for 2 min. The organic layer was separated and filtered through a silica-gel column (Sep-Pack plus), the column washed with dichloromethane and the collected organic phases evaporated. The identity and purity were checked by LC-MS, >90% of the experiments gave >75% yield as estimated from the ELS measurement.

Results

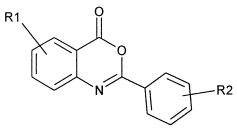
The compounds were evaluated for inhibitory activity on the TF/FVIIa-induced activation of FX using a two-step amidolytic assay. Compounds showing good inhibitory activity in that assay were further tested for activity on FXa and thrombin in order to sort out structures with the best selectivity. Selected compounds were further tested for their ability to prolong clotting time in a standard in vitro clotting assay. Selected data for 2-aryl substituted 4*H*-3,1-benzoxazin-4-ones are depicted in Table 1.

The data revealed a broad variety of selectivity, ranging from compounds having a similar inhibitory effect on



Scheme 2. Solution phase parallel synthesis approach for the preparation of compounds **3**, **4**, **6**–**9**, **12**–**16**, **21**, **25**, **26**, **29**, **31**, **39**, **40**, **42**, **43**, **46**–**50** and **61**.

Table 1. IC_{50} values (μM) for the inhibition of TF/FVIIa-catalyzed activation of FX and the inhibition of FXa and thrombin amidolytic activity



Compound no.	R1	R2	FX activation IC_{50} μM	FXa IC_{50} μM	Thrombin IC_{50} μM
1	H	2-F	4.7	138	56
2	6-Me	2-F	3.6	> 200	145
3	6-CF3	2-F	3.1	37	34
4	6-OMe	2-F	3.0	> 200	40
5	5-Cl,8-Cl	2-F	1.2	180	> 200
6	5-COOMe	2-F	5.6	138	56
7	6-F,7-F	2-F	7.2	> 200	> 200
8	5-NO2	2-F	17	26	17
9	6-F,7-F	2-F,3-F	18	> 200	105
10	6-Br,8-Br	2-F	25	> 200	> 200
11	7-Cl	2-F	30	> 200	199
12	6-Me	2-F,3-F	32	> 200	> 200
13	6-NO2	2-F,3-F	40	NT	NT
14	5-CF3	2-F	> 40	NT	NT
15	8-CF3	2-F	> 40	NT	NT
16	5-COOEt	2-F	> 40	NT	NT
17	5-Cl	2-F,6-F	0.17	4.9	> 200
18	5-NO2	2-F,6-F	0.35	6.3	> 200
19	5-Cl,8-Cl	2-F,6-F	0.36	6.3	> 200
20	6-Me	2-F,6-F	0.60	22	8
21	6-CF3	2-F,6-F	0.63	16	98
22	7-NO2	2-F,6-F	0.82	112	> 200
23	5-F	2-F,6-F	0.90	6	NT
24	6-NO2	2-F,6-F	1.0	19	> 200
25	7-CF3	2-F,6-F	1.4	174	134
26	6-OMe	2-F,6-F	1.5	154	30
27	6-NHAc	2-F,6-F	1.6	35	> 200
28	6-NH2	2-F,6-F	3.2	154	149
29	5-COOMe	2-F,6-F	4.1	112	156
30	5-Me	2-F,6-F	4.3	89	> 200
31	8-CF3	2-F,6-F	4.5	80	> 200
32	7-NH2	2-F,6-F	22	> 200	> 200
33	5-NH2	2-F,6-F	30	> 200	> 200
34	6-Me	2-F,6-Cl	0.9	10	NT
35	5-F	2-F,6-Cl	1.2	6.0	NT
36	6-Me	2-Cl,3-Cl	1.2	110	36
37	6-I	2-Cl	2.2	15	NT
38	6-Me	2-Cl,6-Cl	2.8	63	> 40
39	5-NO2	2-NO2	0.32	< 1.6	97
40	5-NO2	2-OMe	3.9	5.8	19
41	H	2-OMe,5-Cl	6.8	30	NT
42	5-NO2	2-OCOMe	7.3	26	> 200
43	6-NO2	2-OCOMe	10	49	> 200
44	6-Me	2-OCF3	12	> 200	NT
45	6-Cl	2-Br	1.5	6	NT
46	2-Me	2-OCOMe	33	> 200	> 200

all three enzymes to compounds active only in the FX activation assay. Compounds with a detectable inhibitory effect on FXa will also appear to effect FX activation and FVIIa/TF-initiated clot formation, without necessarily inhibiting FVIIa directly. Thus true IC_{50}-values for the FVIIa inhibition as measured by the FX activation assay are obtained only for compounds which do not inhibit Fxa.

The best compounds regarding inhibitory activity and selectivity were **5**, **22** and **25** with 5,8-dichloro, 7-nitro, and 7-trifluoromethyl substitution in the benzoxazine ring, respectively, and 2-fluoro or 2,6-difluoro substitution in the 2-phenyl substituent. These compounds also prolonged the TF-induced clotting time, the ratios being 1.32, 1.31, and 1.51 times the control clotting time. The best inhibitors of the TF/FVIIa-induced activation of FX were substituted with electronegative substituents like Cl, NO_2, CF_3, F in the 5, 6, or 7 positions in the benzoxazine ring together with a 2,6-difluoro substitution in the 2-phenyl group. However, these compounds also possessed good inhibitory effects on the FXa amidolytic activity resulting in a specificity factor below 50, while their activities at the active site of thrombin were low. Also a 6-methyl substituent (compound **20**) inhibited the FX activation at sub-μM concentrations, however, the inhibitory effect on thrombin was also good (8 μM). Compounds carrying-NH_2,-OMe or-COOMe in the benzoxazine ring (compounds **4**, **26**, **28**, **32** and **33**) gave a 3-fold to 10-fold higher IC_{50} in the FX activation assay than found for the electronegative substituents, while the IC_{50} for FXa and thrombin was often found to be > 200 μM (i.e. no effect).

Selected data for the inhibitory effect of 2-heteroaryl substituted 4*H*-3,1-benzoxazin-4-ones is presented in Table 2.

The compounds having a heteroaromatic substituent in position 2 by and large showed a 10-fold higher IC_{50} value than found with a substituted phenyl group in position 2. This probably reflects the difference in electronegativity for the two substituent groups.

Compound **47**, however, had a good profile in agreement with the 6,7-halogen disubstituted 2-phenyl compounds **7**, **9** and **10**.

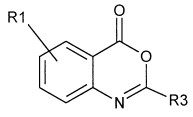
The compounds giving the best prolongation in the clotting time (**17**, **18**, **20**, **28** and **47**) were substituted in the 2-position with a 2,6-difluorophenyl group (or a 2-chloro-3-pyridyl for compound **47**), while the substitution on the benzoxazin ring was Cl, NO_2, Me, NH_2 and F (Table 3).

Discussion

The expected mechanism of action for the serine protease inhibition by benzoxazinone-type of compounds is deactivation by means of reaction of the benzoxazinone lactone with the active site serine, probably under the formation of a drug-enzyme ester followed by its hydrolysis.[7,14] This mechanism was supported by data obtained for activity in a TF/FVII assay and in a FVII amidolytic assay in which we found that the benzoxazinones gave inhibition of the same order of magnitude as found in the FX activation assay (not shown).

The corresponding open anthranilic acid amide derivatives (the 'open form' of the benzoxazinone ring) were found without inhibitory effects. These compounds could be prepared from the corresponding benzoxazinones by hydrolysis with NaOH in methanol followed by careful acidification with an equimolar amount of HCl at room

Table 2. IC_{50} values (μM) for the inhibition of TF/FVIIa-catalyzed activation of FX, FXa and thrombin



Compound no.	R1	R3	FX-activation IC_{50} μM	FX amidolytic IC_{50} μM	Thrombin IC_{50} μM
47	6-F,7-F	2-Cl-3-pyridyl	5.0	> 200	> 200
48	5-Me	2-Cl-3-pyridyl	7.1	> 200	30
49	5-NO2	2-Cl-3-pyridyl	14	98	9.7
50	6-NO2	2-Cl-3-pyridyl	21	140	> 200
51	6-Br6-OMe	4-pyridyl	27	> 200	NT
52	7-OMe	3-Cl,5-CF3-2-pyridyl	33	> 200	NT
53	6-Br	3-Cl,5-CF3-2-pyridyl	33	> 200	NT
54	7-Cl6-OMe	5-NO2-2-furanyl	16	> 200	NT
55	7-Ome	2-furanyl	25	> 200	> 200
56	6-Br	2-furanyl	34	> 200	NT
57	6-Me	5-NO2-2-furanyl	> 40	NT	NT
58	H	2-thienyl	27	> 200	NT
59	6-Me	2-thienyl	35	> 200	> 200
60	6-Me	3-Br-2-thienyl	40	NT	NT
61	6-F,7-F	2-thienyl	> 40	Nt	NT

temperature. Prolonged standing or higher temperatures gave a mixture of the 'open form' and the benzoxazinone. The 'open form' was also accessible from the reaction of anthranilic acid and arylcarboxylic acid chloride in pyridine at −30 °C. However, also this method gave mixtures of the 'open' and ring-closed forms (data not shown). Taking this expected mechanism into account, it seems reasonable to expect that electronegative substituents on the 2-aryl group as well as on the 5, 6, 7, or 8 positions of the benzoxazinone ring will make the ring carbonyl more reactive towards the serine OH-group of the enzyme.

Compounds without substitution in the 2 or 6-positions in the 2-aryl substituent did not inhibit the TF/FVIIa-catalyzed FX activation at concentrations below 40 μM (data not shown).

Introduction of an ester functionality at the 5-position (compounds **6**, **16** and **29**) or at the 2-position in the 2-phenyl substituent (compounds **42**, **43** and **46**) was performed in order to clarify if this group might, in some way, compete with the ester formation between the serine-OH and the benzoxazinone ring, either by steric hindrance of the drug-enzyme reaction or by participating in the ester formation with the enzyme. These compounds, however, gave results of the same magnitude and selectivity as found for both electronegative and electron-donating substituents at the same position. Consequently, no proof in favour of that hypothesis was obtained.

Compounds substituted with 2-thienyl, 2-furanyl or 2-, 3- or 4-pyridyl in the 2-position of the benzoxazine ring (compounds **47**–**61**) showed the same pattern as found for the 2-phenyl substituted compounds. IC_{50} values of < 10 μM were observed only for **47** and **48** carrying an electronegative *ortho*-substituent in the 2-substituent together with 6,7-difluoro or 5-Me substitution. Compound **49** had a surprisingly good effect on thrombin (9.7 μM), i.e. equipotent for thrombin and FVIIa/TF, while the IC_{50} for inhibition of FXa was 10-fold higher. This is in contrast to the findings for compounds **18**, **39** and **40** for which the inhibitory effects were best in the TF/FVIIa-catalyzed FX activation assay.

Table 3. Effects on TF/FVIIa-induced clotting. The ratio of the clotting times with (330 μM) and without test compound is given

Compound no.	Clotting ratio
1	1.45
2	1.47
3	1.59
5	1.32
7	1.60
17	> 3
18	2.58
19	1.68
20	> 3
22	1.31
25	1.51
28	> 3
33	1.06
38	1.88
40	1.25
42	1.14
43	1.22
47	2.32
49	1.52
50	1.16
55	1.35
59	1.76

2-Pyridyl and 4-pyridyl substituents were found to be of low activity in the activation assay, in the former case probably because the ring nitrogen occupied an *ortho* position and no electron-attracting groups were positioned in an '*ortho*' position, and in the latter because no ortho electronegative substituent was present. For the thienyl and furanyl substituted compounds, only compound **60** had an electronegative substituent in the ortho position, however, in this case a bromo substituent was no good compared to compound **45** (IC_{50} values in the FX activation assay being 40 and 1.5 μM, respectively).

Table 4. Analytical data for compounds prepared by solution phase parallel synthesis

No.	Compound	Analytical details[a]
3	2-(2-Fluoro-phenyl)-6-trifluoromethyl-4-*H*-3,1-benzoxazin-4-one	*m/z*[a]: 310 RT: 15.48, ELS: 1%
4	2-(2-Fluoro-phenyl)-6-methoxy-4-*H*-3,1-benzoxazin-4-one	*m/z*: 272 RT: 14.10, ELS: 8%
6	2-(2-Fluoro-phenyl)- 4-*H*-3,1-benzoxazin-4-oxo-5-carboxylic acid methyl ester	*m/z*: 300 RT: 12.97, ELS: 00%
7	6,7-Difluoro-2-(2-fluoro-phenyl)- 4-*H*-3,1-benzoxazin-4-one	*m/z*: 278 RT: 14.78, ELS: 97%
8	2-(2,3-Difluoro-phenyl)-5-nitro-4-*H*-3,1-benzoxazin-4-one	*m/z*: 305 RT: 13.70, ELS: 95%
9	2-(2,3-Difluoro-phenyl)-6,7-difluoro-4-*H*-3,1-benzoxazin-4-one	*m/z*: 296 RT: 14.91, ELS: 98%
12	2-(2,3-Difluoro-phenyl)-5-methyl-4-*H*-3,1-benzoxazin-4-one	*m/z*: 274 RT: 15.1, ELS: 82%
13	2-(2,3-Difluoro-phenyl)-6-nitro-4-*H*-3,1-benzoxazin-4-one	*m/z*: 305 RT: 14.26, ELS: 80%
14	2-(2-Fluoro-phenyl)-5-trifluoromethyl-4-*H*-3,1-benzoxazin-4-one	*m/z*: 310 RT: 15.47, ELS: 81%
15	2-(2-Fluoro-phenyl)-8-trifluoromethyl-4-*H*-3,1-benzoxazin-4-one	*m/z*: 310 RT: 15.15, ELS: 99%
16	2-(2-Fluoro-phenyl)-4-oxo-4-*H*-3,1-benzoxazin- 5-carboxylic acid ethyl ester	*m/z*: 314 RT: 13.73, ELS: 95%
21	2-(2,6-Difluoro-phenyl)-6-trifluoromethyl-4-*H*-3,1-benzoxazin-4-one	*m/z*: 328 RT: 15.26, ELS: 82%
25	2-(2,6-Difluoro-phenyl)-7-trifluoromethyl-4-*H*-3,1-benzoxazin-4-one	*m/z*: 328 RT: 15.51, ELS: 91%
26	2-(2,6-Difluoro-phenyl)-6-methoxy-4-*H*-3,1-benzoxazin-4-one	*m/z*: 290 RT: 13.8/11.45, ELS: 80%
29	2-(2,6-Difluoro-phenyl)-4-oxo-4-*H*-3,1-benzoxazin-5-carboxylic acid methyl ester	*m/z*: 318 RT: 12.82, ELS: 67%
31	2-(2,6-Difluoro-phenyl)-8-trifluoromethyl-4-*H*-3,1-benzoxazin-4-one	*m/z*: 328 RT: 14.73, ELS: 94%
39	5-Nitro-2-(2-nitro-phenyl)- 4-*H*-3,1-benzoxazin-4-one	*m/z*: 314 RT: 13.11, ELS: 81%
40	2-(2-Methoxy-phenyl)-5-nitro-4-*H*-3,1-benzoxazin-4-one	*m/z*: 299 RT: 12.81, ELS: 61%
42	Acetic acid 2-(5-nitro-4-oxo-4-*H*-3,1-benzoxazin-2-yl)-phenyl ester	*m/z*: 327 RT: 12.88, ELS: 84%
43	Acetic acid 2-(6-nitro-4-oxo-4-*H*-3,1-benzoxazin- 2-yl)-phenyl ester	*m/z*: 327 RT: 13.37, ELS: 54%
46	Acetic acid 2-(5-methyl-4-oxo-4-*H*-3,1-benzoxazin-2-yl)-phenyl ester	*m/z*: 296 RT: 14.11, ELS: 54%
47	2-(2-Chloro-pyridin-3-yl)-6,7-difluoro-4-*H*-3,1-benzoxazin-4-one	*m/z*: 295 RT: 12.75, ELS: 96%
48	2-(2-Chloro-pyridin-3-yl)-5-methyl-4-*H*-3,1-benzoxazin-4-one	*m/z*: 273 RT: 12.61, ELS: 100%
49	2-(2-Chloro-pyridin-3-yl)-5-nitro-4-*H*-3,1-benzoxazin-4-one	*m/z*: 304 RT: 11.64, ELS: 82%
50	2-(2-Chloro-pyridin-3-yl)-6-nitro-4-*H*-3,1-benzoxazin-4-one	*m/z*: 304 RT: 11.94, ELS: 88%
61	6,7-Difluoro-2-thiophen-2-yl-4-*H*-3,1-benzoxazin-4-one	*m/z*: 266 RT: 14.68, ELS: 98%

[a]*m/z*: The molecular ion (M + 1) from the LC-MS investigation; RT: Retention time (min); ELS: The purity estimated from the electrospray (positive ion) measurement.

Conclusion

The synthetic approach described easily yielded a series of 2-aryl substituted 4*H*-3,1-benzoxazin-4-ones in fair to good yields by standard synthetic methods and through a solution-phase parallel fashion. The 2-aryl substituted 4*H*-3,1-benzoxazin-4-ones described comprise several compounds with excellent activity and selectivity thus being the first small molecules described having that profile. Good inhibition requires the presence of at least one electron-attracting substituent in the *ortho* position of the 2-aryl or heteroaryl substituent, the best structures also bearing one or two electronegative substituents in the 5, 6, 7 or 8 positions. The best compounds as regards both inhibitory activity of the TF/FVIIa-catalyzed FX activation and best selectivity (**5**, **22** and **25**) were all found within this pattern. Simple heterocycles like thienyl, furanyl and 2- and 4-pyridyl as the 2-substituents gave compounds of low activity while 2-(3-pyridyl) substituents were found to be of similar activity as the phenyl substituents (**47**).

Experimental

General procedures. Melting points (uncorrected) were measured on a Büchi 535. NMR spectra were recorded on a Bruker AVANCE DPX 200 Mhz or 300 MHz instrument operating at room temperature. Mass spectra were obtained on a Finnigan Mat TSQ 70 apparatus using a direct inlet system. The HPLC-MS analyses were performed on a PE Sciex API 100 LC/MS System using a WatersTM 3 mm×150 mm, 3.5 μ, C-18 Symmetry column and positive ion spray with a flow rate at 20 μL/min. The column was eluted with a linear gradient of 5–90% A, 85–0% B and 10% C for 15 min at a flow rate of 1 mL/min (solvent A = acetonitrile, solvent B = water and solvent, C = 0.1% trifluoroacetic acid in water). Microanalyses were performed by Novo Nordisk Analytical Department.

FX activation assay

The compounds were dissolved in DMSO and mixed with FVIIa (produced in-house) in 50 mM Hepes, pH 7.4, containing 0.1 M NaCl, 5 mM $CaCl_2$, and 1 mg/mL bovine serum albumin (1 + 5). 30 μL of this mixture was then mixed with 45 μL TF (American Diagnostica, relipidated in PC/PS vesicles) and 25 μL of FX (Enzyme Research Laboratories), all in a Ca^{2+}-containing buffer. This gave final concentrations of 100 pM FVIIa, 5 pM TF, 175 nM FX, and various concentrations of the compounds. After a 5 min incubation, the FVIIa/TF-catalyzed activation of FX was terminated by an addition of a 50 μL buffer containing enough EDTA to give an excess over the Ca^{2+} ions present. 50 μL of a 2-mM solution of S-2765 (Chromogenix, 2 mM in water) was then added and the FXa formed was allowed to hydrolyze the substrate for 10 min during which the absorbance at 405 nM was continuously monitored in a SPECTRAmax™ 340 plate reader. The slope of the absorbance curve was compared to that of a control where only DMSO was added to FVIIa/TF/FX.

Thrombin activity assay

170 μL thrombin (Boehringer Mannheim, 3.5 nM) in the buffer described above, 10 μL test compound in DMSO (or DMSO alone in the control) and 20 μL

S-2238 (Chromogenix, 10 mM in water) were mixed. The hydrolysis of S-2238 was monitored as described for the FX activation assay.

FXa activity assay

170 μL FXa (Enzyme Research Laboratories, 1.2 nM in buffer) in the buffer desribed above, 10 μL test compound and 20 μL S-2765 (Chromogenix, 10 mM in water) were mixed. The hydrolysis of S-2765 was monitored as described for the FX activation assay.

FVIIa/TF-initiated clotting assay

The test compounds, 20 mM in DMSO, were diluted in citrated normal human plasma just before the analysis (1 + 19) and placed in the sample carousel of an ACL 300 Research coagulometer. 55 μL sample (compound in plasma) was mixed with 55 μL of thromboplastin (Innovin, Dade; diluted 1:500 after dissolution) and incubated for 5 min. The clotting reaction was started by adding 55 μL of a 25 mM $CaCl_2$ solution, yielding a final compound concentration of 0.33 mM. The ratio between the clotting time in the presence and absence of test compounds was used to quantify the anticoagulant effect.

The following compounds were commercially available from Maybridge (compounds **1**, **10**, **11**, **23**, **34**, **35**, **37**, **51**, **52**, **53**, **54**, **57** and **58**), CSC (compounds **41**, **55** and **56**), and SPECS (compound **45**).

2-(2-Fluoro-phenyl)-6-methyl-4-*H*-3,1-benzoxazin-4-one (2). 2-Fluorobenzoic acid (3.3 mmol, 0.52 g) was mixed with HOBT (3.3 mmol, 0.44 g) and EDAC (6.6 mmol, 1.26 g) in CH_2Cl_2 (5 mL). The mixture was stirred for 1 h and subsequently 2-amino-5-methylbenzoic acid (3.3 mmol, 0.5 g) in DMF (5 mL) was added dropwise (10 min). After stirring for 3 h, HCl (4N, 10 mL) was added and the mixture extracted with CH_2Cl_2 (3×10 mL). The collected organic phases were extracted with HCl (4N, 10 mL) and then twice with water (10 mL). The organic phases were dried ($MgSO_4$), filtered and evaporated to dryness. The crude product was purified on silica-gel using CH_2Cl_2 as eluent. Yield of **2** (30%); LC-MS 256 (M + 1); RT 14.61 min; ELS-purity 100%; mp 144 °C. Calculated for $C_{15}H_{10}FNO_2$: C, 70.58%; H, 3.95%; N, 5.49%. Found: C, 70.41%; H, 3.98%; N, 5.39%. ^{1}H NMR ($CDCl_3$, ppm) 8.08 (1H, d t), 7.98 (1H, d), 7.82–7.57 (3H, m), 7.57–7.32 (2H, m), 2.45 (3H, s).

5,8-Dichloro-2-(2-fluoro-phenyl)-4*H*-3,1-benzoxazin-4-one (5). 2-Amino-3,6-dichlorobenzoic acid (0.5 g) and triethyl:toluene (1:1) (20 mL) were mixed. 2-Fluorobenzoyl chloride (0.77 g) was added dropwise under cooling and stirring. The mixture was subsequently heated to room temperature and stirred until the disappearance of the starting material was observed on TLC (silica-gel) using heptane:ethyl acetate (4:1) as eluent. After cooling on ice, the precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was purified by column chromatography on silica-gel using heptane:ethyl acetate (4:1) as eluent. Yield 0.31 g (42%); mp 140 °C; MS 311/309 (M^+) (Cl isotope pattern). Calculated for $C_{14}H_6Cl_2FNO_2$: C, 54.22%; H, 1.95%; N, 4.52%. Found: C, 53.53%; H, 1.91%; N, 4.38%. ^{1}H NMR ($CDCl_3$, ppm) 8.20 (1H, double t); 7.78 (1H, d); 7.65–7.52 (1H, m); 7.48 (1H, d); 7.35–7.15 (2H, m).

5-Chloro-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (17). 6-Chloroanthranilic acid (0.566 g), 2,6-difluorobenzoyl chloride (0.93 mL) and triethyl amine:toluene (1/1) (18 mL) were reacted by heating to 50 °C for 2 days. Extraction between ethyl acetate (100 mL) and HCl (2N, 100 mL), followed by separation of the organic layer, drying over $MgSO_4$, and finally filtering and evaporation gave a crude product that was dissolved in warm THF (20 mL) and precipitated with hexane. The resulting mixture was further purified on a silica-gel column using dichloromethane as eluent. The isolated fraction was dissolved in warm THF (20 mL) and precipitated with hexane, yielding **17** (0.65 g, 70%); mp. 176 °C; MS *m*/*e* 293/295 (M^+). Calculated for $C_{14}H_6Cl_2FNO_2$: C, 57.62%; H, 2.06%; N, 4.77%. Found: C, 57.35%; H, 2.06%; N, 4.60%. ^{1}H NMR (DMSO, ppm) 7.93 (1H, t); 7.82–7.65 (3H, m); 7.38 (1H, d); 7.32 (1H, d).

2-(2,6-Difluoro-phenyl)-5-nitro-4-*H*-3,1-benzoxazin-4-one (18). 6-Nitroanthranilic acid (0.6 g), 2,6-difluorobenzoyl chloride (0.93 mL) and triethyl amine:toluene (1:1) (18 mL) were reacted by heating to 50 °C for 2 days. Extraction between ethyl acetate (100 mL) and HCl (2N, 100 mL), followed by separation of the organic layer, drying over $MgSO_4$, and finally filtering and evaporation gave a crude product that was dissolved in warm THF (20 mL) and precipitated with hexane. The resulting mixture was further purified on a silica-gel column using dichloromethane as eluent. The isolated fraction was dissolved in warm THF (20 mL) and precipitated with hexane, yielding **18** (0.475 g, 40%); mp 172 °C; MS *m*/*e* 304 (M^+); LC-MS *m*/*e* 305 (M + 1); ELS-purity 100%. $C_{14}H_6F_2N_2O_4$: C, 56.25%; H, 2.02%; N, 7.03%. Found: C, 55.27%; H, 1.99%; N, 8.96%. ^{1}H NMR (DMSO, ppm) 8.22–8.05 (2H, m); 7.97 (1H, dd); 7.85–7.68 (1H, m) 7.36 (2H, t).

5,8-Dichloro-2-(2,6-difluoro-phenyl)- 4-*H*-3,1-benzoxazin-4-one (19). 2-Amino-3,6-dichlorobenzoic acid (0.125 g), 2,6-difluorobenzoyl chloride (0.168 mL) and triethyl amine:toluene (1:1) (1,5 mL) were reacted by heating to 50 °C for 2 h. HCL (0.2N, 1 mL) was added and the organic layer separated and rinsed by pressing through a silica-gel column (Sep Pack). Evaporation resulted in the isolation of **19** (0.20 g, 10%); mp 190 °C; MS *m*/*e* 328 (M^+); LC-MS 329 (M + 1); RT 14.85; ELS-purity 99,1%. $C_{14}H_{5)}Cl_2F_2NO_2$: C, 51.25%; H, 1.54%; N, 4.27%. Found: C, 51.69%; H, 1.95%; N, 4.02%. ^{1}H NMR (DMSO, ppm) 8.08 (1H, d); 7.88–7.68 (2H, m); 7.35 (2H, t).

2-(2,6-Difluoro-phenyl)-6-methyl-4*H*-3,1-benzoxazin-4-one (20). 2-Amino-5-methylbenzoic acid (0.5 g) and triethyl amine (10 mL) were mixed in dry toluene (20 mL). 2,6-Difluorobenzoyl chloride (1.28 g) was added dropwise

under cooling and stirring. The mixture was subsequently heated to room temperature for 2 days. After cooling on ice, the precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was purified by column chromatography on silica-gel using heptane/ethyl acetate as eluent. Yield of **20** (0.85 g, 95%); mp 156 °C; LC-MS 274 (M + 1); RT 14.22 min; ELS-purity 100%. $C_{15}H_9F_2NO_2$: C, 65.69%; H, 3.30%; N, 5.15%. Found: C, 66.18%; H, 3.33%; N, 5.06%. ^{1}H NMR (DMSO, ppm) 8.03 (1H, d); 7.90–7.60 (3H, m); 7.35 (2H, t); 2.50 (3H, s).

2-(2,6-Difluoro-phenyl)-7-nitro-4-*H*-3,1-benzoxazin-4-one (22). 4-Nitroanthranilic acid (0.6 g), 2,6-difluorobenzoyl chloride (0.93 mL) and triethyl amine:toluene (1:1) (18 mL) were reacted by heating to 50 °C for 2 days. Extraction between ethyl acetate (100 mL) and HCl (2N, 100 mL), followed by separation of the organic layer, drying over $MgSO_4$, and finally filtering and evaporation gave a crude product which was dissolved in warm THF (20 mL) and precipitated with hexane twice to get the pure product **22** (0.42 g, 41%); mp 187 °C; MS 304 (M^+). $C_{14}H_6F_2N_2O_4$: C, 55.28%; H, 1.99%; N, 9.21%. Found: C, 54.85%; H, 2.07%; N, 9.39%. ^{1}H NMR (DMSO, ppm) 8.38 (3H, s, broad); 7.88–7.68 (1H, m); 7.35(2H, t).

2-(2,6-Difluoro-phenyl)-6-nitro-4-*H*-3,1-benzoxazin-4-one (24). 5-Nitroanthranilic acid (0.6 g) was dissolved in triethyl amine:toluene (1:1) (18 mL) and stirred for 10 min. 2,6-Difluorobenzoyl chloride (1.3 g) was slowly added under stirring resulting in the formation of a precipitate. The reaction was performed in an N_2-atmosphere. After stirring at room temperature for 24 h, the mixture was extracted with saturated $NaHCO_3$ and ethyl acetate (20 mL), and the organic layer was separated and evaporated. The crude product was rinsed by precipitation from hot dioxane, the resulting mass transferred to a silica-gel cloumn and eluted with dichloromethane, the isolated fraction was dissolved in hot toluene (2 mL) and precipitated with hexane, resulting in **24** (0.13 g, 13%); mp 188 °C; MS 304 (M^+). $C_{14}H_6F_2N_2O_4$: C, 55.28%; H, 1.99%; N, 9.21%. Found: C, 52.41%; H, 1.91%; N, 8.55%. ^{1}H NMR (DMSO, ppm) 8.81 (1H, dd); 8.70 (1H, dd); 7.95 (1H, d); 7.86–7.68 (1H, m); 7.35 (2H, t).

6-Acetamido-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (27). 5-Acetamidoanthranilic acid (0.64 g) and 2,6-difluorobenzoyl chloride (1.3 g) were reacted in triethyl amine:toluene (1:1) (20 mL) by heating to 50 °C for 1 h. Ethyl acetate (50 mL) and HCl-solution (1 mL 4N in 50 mL water) were added resulting in the formation of a precipitate. The mixture was filtered and the residue dissolved in THF followed by evaporation to dryness, subsequent dissolution in hot dioxane followed by precipitation with hexane resulted in colourless crystals of **27** (0.98 g, 95%); mp 246 °C; MS *m/e* 316 (M^+); LC-MS 317 (M + 1); RT 10.32; ELS-purity 100%. $C_{16}H_{10}F_2N_2O_3$: C, 60.76%; H, 3.19%; N, 8.86%. Found: C, 60.31%; H, 3.19%; N, 8.44%. ^{1}H NMR (DMSO, ppm) 8.53 (1H, dd); 8.04 (1H, dd); 7.72 (2H, dd); 7.35 (2H, t).

6-Amino-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (28). 6-Nitro-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one **24** (50 mg) was dissolved in acetic acid (5 mL) under N_2, PtO_2 (2.5 mg) was added and the mixture was hydrogenated with H_2. Reaction time 2 h. The reaction mixture was filtered through Hyflo® which was rinsed afterwards with ethyl acetate. The combined organic phases were evaporated to dryness and subsequently treated three times with toluene followed by evaporation. The resulting mixture was dissolved in THF and precipitated with hexane resulting in **28** (12 mg, 20%); LC-MS 275 (M + 1); RT 10.28 min; ELS-purity 96%. ^{1}H NMR (MeOH, ppm) 7.70–7.50 (1H, m); 7.45 (1H, d); 7.36 (1H, d); 7.26–7.08 (3H, m).

2-(2,6-Difluoro-phenyl)-5-methyl-4-*H*-3,1-benzoxazin-4-one (30). 2-Amino-6-methylbenzoic acid (0.498 g), 2,6-difluorobenzoyl chloride (0.93 mL) and triethyl amine: toluene (1:1) (20 mL) were reacted by heating to 50 °C for 2 h. Extraction between ethyl acetate (100 mL) and HCl (2N, 100 mL), followed by separation of the organic layer, drying over $MgSO_4$, and finally filtering and evaporation gave a crude product which was re-dissolved in toluene (10 mL) and precipitated with hexane (5 mL) resulting in **30** (0.45 g, 50%); mp 156 °C; MS 273 (M^+); LC-MS 274 (M + 1); RT 14.34 min; ELS-purity 100%. $C_{15}H_9F_2NO_2$: C, 65.94%; H, 3.32%; N, 5.13%. Found: C, 65.24%; H, 3.27%; N, 4.85%. ^{1}H NMR (DMSO, ppm) 7.90–7.67 (2H, m); 7.55 (2H, t); 7.35 (2H, t).

7-Amino-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (32). 7-Nitro-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one **22** (0.10 g) was dissolved in acetic acid (10 mL) under N_2, PtO_2 (5 mg) was added and the mixture was hydrogenated with H_2 gas. Reaction time day. The reaction mixture was filtered through Hyflo®, which was rinsed afterwards with ethyl acetate. The combined organic phases were evaporated to dryness and subsequently treated three times with toluene followed by evaporation. The resulting mixture was purified on a silica-gel column using dichloromethane as eluent. One fraction was collected and identified as **32** (15 mg, 20%); mp 196 °C; MS 274 (M^+). $C_{14}H_8F_2N_2O_2$: C, 61.32%; H, 2.94%; N, 10.22%. Found: C, 61.44%; H, 3.45%; N, 8.91%. ^{1}H NMR (DMSO, ppm) 7.82 (1H, d); 7.70–7.58 (1H, m); 7.35 (2H, t); 6.85 (1H, dd); 6.75 (2H, s, broad, NH); 6.68 (1H, d).

5-Amino-2-(2,6-difluoro-phenyl)-4-*H*-3,1-benzoxazin-4-one (33). 5-Nitro-2-(2,6-difluoro-phenyl)-4-H-3,1-benzoxazin-4-one **18** (0.4 g) was dissolved in acetic acid (30 mL) under N_2, PtO_2 (20 mg) was added and the mixture was hydrogenated with H_2. Reaction time 1 day. The reaction mixture was filtered through Hyflo®, which was rinsed afterwards with ethyl acetate. The combined organic phases were evaporated to dryness and subsequently treated 3 times with toluene followed by evaporation. The resulting mixture was dissolved in THF and precipitated with hexane giving a crude product that was purified on a silica-gel column using dichloromethane as eluent. One fraction was collected

(51 mg), identified as **33**; LC-MS 275 (M + 1); RT 12.45 min; ELS-purity 100%; mp 197 °C. $C_{14}H_8F_2N_2O_2$: C, 61.32%; H, 2.94%; N, 10.22%. Found: C, 61.55%; H, 3.04%; N, 9.73%. ^{1}H NMR (CDCl3, ppm) 7.55–7.38 (2H, m); 7.05 (2H, t); 6.90 (1H, d); 6.70 (1H, d); 6.08 (2H, s, broad, NH).

2-(2,3-Dichloro-phenyl)-6-methyl-4-*H*-3,1-benzoxazin-4-one (36). 2-Amino-5-methylbenzoic acid (0.5 g) and dry pyridine (20 mL) were mixed with 2,3-dichlorobenzoyl chloride (1.52 g) in dry toluene (20 mL) at room temperature. Subsequently, the mixture was heated to 80 °C for 1 h. The mixture was evaporated to dryness, and the residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was recrystallised from toluene yielding **36** (1.0 g, 99%); mp 166 °C. $C_{15}H_9Cl_2NO_2$: C, 58.85%; H, 2.96%; N, 4.58%. Found: C, 58.88%; H, 2.96%; N, 4.45%. ^{1}H NMR (CDCl3, ppm) 7.55–7.38 (2H, m); 7.05 (2H, t); 6.90 (1H, d); 6.70 (1H, d); 6.08 (2H, s, broad, NH).

2-(2,6-Dichloro-phenyl)-6-methyl-4-*H*-3,1-benzoxazin-4-one (38). 2-Amino-5-methylbenzoic acid (0.5 g) and triethyl amine (10 mL) were mixed in dry toluene (20 mL). 2,6-Dichlorobenzoyl chloride (1.52 g) was added dropwise under cooling and stirring. The mixture was subsequently heated to room temperature for 24 h, then heated to 80 °C for 1 h. After cooling on ice, the precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was purified by column chromatography on silica-gel using heptane/ethyl acetate as eluent giving **38** (0.12 g, 11%); mp 175 °C; MS 305, 307 (M^+) (Cl isotope pattern); LC-MS 306, 308 (M + 1); RT 15.75 min; ELS-purity 98%. $C_{15}H_9Cl_2NO_2$: C, 58.85%; H, 2.96%; N, 4.58%. Found: C, 57.96%; H, 2.90%; N, 4.09%. ^{1}H NMR ($CDCl_3$, ppm) 8.10 (1H, s, broad); 7.72–7.58 (2H, m); 7.48–7.31 (3H, m); 2.54 (3H, s).

6-Methyl-2-(2-trifluoromethoxy-phenyl)-4*H*-3,1-benzoxazin-4-one (44). 2-Amino-5-methylbenzoic acid (0.5 g) and triethyl amine (10 mL) were mixed in dry toluene (20 mL). 2-Trifluoromethoxybenzoyl chloride (1.64 g) was added dropwise under cooling and stirring. The mixture was subsequently heated to room temperature for 2 days. After cooling on ice, the precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was purified by column chromatography on silica-gel using heptane/ethyl acetate as eluent giving **44** (1.0 g, 98%); mp 70–80 °C; MS 321 (M^+). $C_{16}H_{10}F_3NO_3$: C, 59.82%; H, 3.14%; N, 4.36%. Found: C, 61.12%; H, 3.53%; N, 4.18%. ^{1}H NMR ($CDCl_3$, ppm) 8.10 (1H, dd); 8.04 (1H, d, broad); 7.70–7.52 (3H, m); 7.49–7.36 (2H, m); 2.50 (3H, s).

6-Methyl-2-thiophen-2-yl-4*H*-3,1-benzoxazin-4-one (59). 2-Amino-5-methylbenzoic acid (1.5 g) and triethyl amine:toluene (1:1) (80 mL) were mixed with 2-thienylcarbonyl chloride (3.18 g). The mixture was stirred at room temperature for 5 h and the precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was purified by dissolution in dioxan followed by precipitation with hexane resulting in **59** (2.0 g, 85%); mp 156 °C; MS 243 (M^+); LC-MS 244 (M + 1); RT 1.61 min; ELS-purity 100%. $C_{13}H_9NO_2S$: C, 64.18%; H, 3.73%; N, 5.76%. Found: C, 64.20%; H, 3.72%; N, 5.56%. ^{1}H NMR (DMSO, ppm) 7.95 (1H, dd); 7.86 (2H, s, broad); 7.70 (1H, dd); 7.50 (1H, dd); 7.27 (1H, t); 2.42 (3H, s). ^{13}C NMR (DMSO, ppm) 159.24, 153.28, 145.02, 139.08, 138.73, 134.65, 134.11, 132.21, 129.58, 128.53, 127.12, 117.12, 21.54.

2-(3-Bromothiophen-2-yl)-6-methyl-4*H*-3,1-benzoxazin-4-one (60). 2-Amino-5-methylbenzoic acid (0.5 g) and triethyl amine (10 mL) were mixed in dry toluene (10 mL). 3-Bromo-2-thienylcarbonyl chloride (0.72 g) was added dropwise under cooling and stirring, the mixture was further stirred at room temperature for 2 days and subsequently the mixture was kept at 4 °C for 30 days. The precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between methylene chloride and potassium carbonate (2N), the organic layers separated, dried over magnesium sulfate and evaporated to dryness. The raw product was purified by column chromatography on silica-gel using toluene as eluent. Yield of **60** (0.45 g, 48%); mp 159 °C; MS 321, 323 (M^+) (Br-isotope pattern). NMR ($CDCl_3$, ppm) 7.99 (1H, dd); 7.66–7.50 (2H, m); 7.49 (1H, d); 7.12 (1H, d); 2.47 (3H, s). ^{13}C NMR ($CDCl_3$, ppm) 159.13, 152.37, 144.93, 139.31, 138.28, 134.15, 131.18, 128.88, 128.74, 127.36, 116.91, 115.14, 21.75.

The following compounds were prepared using solution phase parallel synthesis following the general description below:

The appropriately substituted 2-aminobenzoic acid (30 mg) was dissolved in toluene (300 uL), triethyl amine (300 uL) was added and the mixture shaken for 10 min at room temperature. Appropriately substituted benzoic acid chloride (2.2 equivalent) was added and the mixture shaken overnight at room temperature. Ethyl acetate (1 mL) and HCl (0.1N, 1 mL) were added followed by shaking for 30 min. The organic layer was separated and saturated, $NaHCO_3$ (0.5 mL) added followed by shaking for 2 min. The organic layer was separated and filtered through a silica-gel column (Sep-Pack plus), the column was washed with dichloromethane (3 mL) and the collected organic phases evaporated. The identity and purity was checked by LC-MS using a PE Sciex API 100 LC/MS system using Waters 3 mm × 150 mm 3.5 u C-18 symmetry column and positive ion-spray with a flow rate at 20 uL/min (Table 4).

References

1. Rapaport, S. I.; Rao, L. V. M. *Thromb. Haemost*. **1995**, *74*, 7.
2. Harker, L. A.; Hansen, S. R.; Kelly, A. B. *Thromb. Haemost*. **1995**, *74*, 464.
3. Papadopopoulos, E. P. *Heterocycles* **1982**, *6*, 1039.
4. Hamprecht, G.; Wuerzer, B. *US patent* **1982**, *4*, 315, 766.
5. Johnson, J. L.; Pattison, I. *J. Heterocycl. Chem*. **1986**, *23*, 249.
6. Fenton, G. *J. Med. Chem*. **1989**, *32*, 265.
7. Krantz, A. *J. Med. Chem*. **1990**, *33*, 464.
8. Rose, U. *J. Heterocyclic Chem*. **1991**, *28*, 2005.
9. Gilmore, J. L. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 679.
10. Jarvest, R. L. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 2463.
11. Hays, S. J. *J. Med. Chem*. **1998**, *41*, 1060.
12. Abood, N. A. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 2105.
13. Gordeev, M.; Patel, D. *WO patent*, **1998**, *98*, 33783.
14. Neumann, U.; Gütschow, M. *Bioorg. Chem*. **1995**, *23*, 72.