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Selective and orally bioavailable phenylglycine tissue factor/factor VIIa inhibitors

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Abstract—We describe the structure-based design and synthesis of highly potent, orally bioavailable tissue factor/factor VIIa inhibitors which interfere with the coagulation cascade by selective inhibition of the extrinsic pathway.

Thromboembolic disorders are among the leading causes for morbidity and mortality in the industrialized world. Thrombosis of either the venous or the arterial vascular system may cause pulmonary embolism, myocardial infarction or ischemic stroke.[1] The development of new anticoagulant therapies therefore represents an important medical need. For a long period of time the coumarins have been the only orally active anticoagulants interfering with the coagulation cascade which have been available on the market. They act indirectly, inhibiting the γ-carboxylation in the biosynthesis of coagulation factors prothrombin, VII, IX, and X. This mechanism leads to a slow on-set of action. Additionally, the coumarins suffer from substantial food and drug interaction, and high protein binding,[2] rendering it very difficult to maintain a balanced plasma exposure. Therefore, careful and regular monitoring of the patient is required.[3] During the last decades, substantial efforts have been devoted to the search for an orally bioavailable replacement of the coumarins. Out of numerous research programs, the only compound to have reached the market is Ximelagatran from AstraZeneca, which is a double prodrug of the direct thrombin inhibitor Melagatran.[4] Ximelagatran has been launched in France in 2004 under the tradename Exanta, but so far has not been accepted by the FDA due to concerns about long-term liver damage and possible risk of heart attacks.

It is known that excessive inhibition of the coagulation cascade at the final stages (factor Xa, thrombin) can lead to bleeding complications.[5] An alternative target would be the inhibition of the tissue factor/factor VIIa (TF/F.VIIa) complex which is the main trigger of thrombotic events.[6] This complex is part of the extrinsic pathway of the coagulation cascade and causes the activation of factors IX and X, ultimately resulting in the generation of thrombin and the thrombin-mediated conversion of fibrinogen to fibrin. Proof-of-concept experiments in animal models from our laboratories[7] as well as other research groups[8] demonstrate that specific inhibition of the TF/F.VIIa complex results in an antithrombotic effect without enhancing bleeding propensity. These results suggest that F.VIIa is a very promising target for a novel anticoagulant, and many research programs in the pharmaceutical industry aim at the discovery of a low molecular weight TF/F.VIIa inhibitor.[9] Here, we report the design, synthesis, and profiling of the first highly potent, orally bioavailable TF/F.VIIa inhibitors from our laboratories.

Results and Discussion. In a previous publication[10], compounds **1**–**3** (Table 1) were described. They were the first TF/F.VIIa inhibitors discovered in our laboratories exhibiting an activity in the single digit nanomolar range. In addition to their good inhibitory activity,

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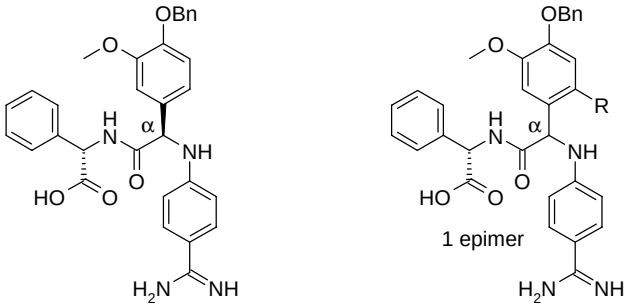
Table 1. Inhibition of TF/F.VIIa and related serine proteases by low nanomolar compounds **1**–**3**



Compound	K_i (μM)				Thrombin	$2 \times PT^a$ (μM)
	F.VIIa	Thrombin	F.Xa	Trypsin	F.VIIa	
1	0.004	0.035	8.0	0.04	8.8	4
2	0.002	0.54	12.4	0.05	270	12
3	0.007	6.4	19.0	0.08	913	44

[a] Human citrated plasma is spiked with at least six concentrations of inhibitor. Clotting is initiated by addition of exogenous tissue factor (Innovin). Clotting time is determined by a turbidity measurement. The concentration of inhibitor necessary to double control clotting time is determined by fitting the data to an exponential regression.[11]

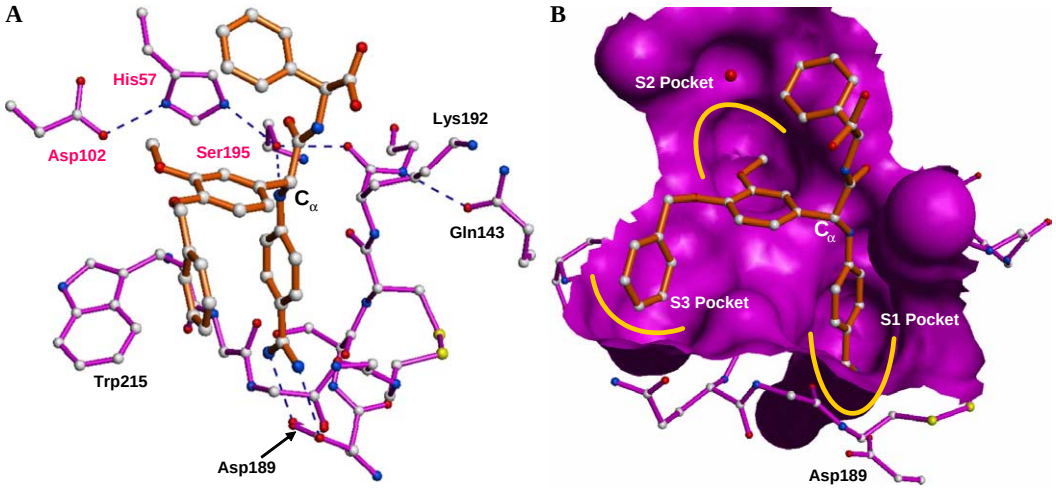


Figure 1. Crystal structure (1w7x.pdb) of compound **1** bound to the active site of F.VIIa. (A) Ball-and-stick representation. (B) Surface representation. Crystals of compound **1** complexed with TF/F.VIIa were originally produced as described.[10] Crystals of the complex of **1** with short form recombinant F.VIIa were produced as described[12], except that the enzyme was activated before complex formation. Crystals were measured on the Swiss Norwegian Beam Lines (SNBL) at the ESRF, Grenoble, to resolutions of 2.5 Å and 2.85 Å, respectively. The inhibitor binding mode could be determined in both cases, but the data sets were incomplete and difficult to refine satisfactorily. In the complex with short form recombinant F.VIIa, the inhibitor binding is disturbed by a crystal contact. Nevertheless, this structure without TF has been deposited in the Protein Data Bank as 1w8b.pdb. Recently, the structure determination in a new crystal form and at high resolution has been repeated for this publication. To this end crystals of short form recombinant human F.VIIa were prepared as described.[13] Crystals of the complex with **1** were frozen and data were measured in-house to 1.8 Å resolution and processed with XDS.[14] The unit cell is P41212, with $a = b = 95.0$ Å, and $c = 115.85$Å. The structure was refined, starting from coordinates of other in-house structures, using Refmac5[15] and ARP/wARP[16], to final overall crystallographic *R*-factors of 16.9% (working) and 19.5% (free), with values in the outer shell of 25.8% and 27.7%, respectively. The inhibitor density is clear. Coordinates of this new short form recombinant human F.VIIa complex have been deposited in the Protein Data Bank as 1w7x.pdb. An unexpected observation is that the peptide bond between Lys192 and Gly193 is flipped by 180° relative to its usual 'active' conformation into the 'inactive' conformation shown. The carbonyl oxygen of Lys192 is hydrogen bonded to the –OH of Ser195 and the –NH of Gly193, which normally donates a hydrogen bond as part of the oxyanion-hole, now donates a hydrogen bond to the side-chain oxygen of Gln143. Factor VIIa is unique among the related serine proteases in having a glutamine in position 143 which can turn inwards to stabilize the 'inactive' conformation. This 'inactive' conformation is seen both in the presence and absence of tissue factor for this inhibitor, although in the complex with tissue factor the loop containing Gln143 is poorly ordered. We speculate that the 'inactive' conformation is the more stable conformation in the factor VIIa/tissue factor complex and that factor X, the canonical macromolecular substrate, must expend some binding energy to promote the active conformation. Such an 'inactive' conformation was described in the literature[17] for the epidermolytic toxin A, a functionally unrelated serine protease, including an estimate of the energy required to flip the peptide. In our hands, some inhibitor series bind to the 'inactive' conformation and others to the 'active' conformation (unpublished results).

compounds **2** and **3** showed promising selectivity against thrombin and factor Xa (F.Xa). The drawback of these selective TF/F.VIIa inhibitors is their insufficient PK profile. Additionally, their poor physicochemical behavior and their high molecular weight cause a moderate functional activity in the PT (prothrombin time) assay which is a measure of the ability of an inhibitor to prevent clotting via the extrinsic pathway of the coagulation cascade.[11] In this report, we describe how we were able to reduce the molecular weight and the complexity of the phenylglycine amide TF/F.VIIa inhibitors while maintaining selectivity and improving plasma activity.

The X-ray crystal structure of compound **1** bound to the active site of TF/F.VIIa is shown in Figure 1. The amidine forms the expected salt bridge with the carboxylate of Asp189 at the bottom of the S1 pocket. A hydrogen bond is formed between the aniline NH and the hydroxyl group of the active site serine (Ser195). The methoxy group *meta* to C_α of the phenylglycine moiety is located in the small S2 pocket of F.VIIa. The carboxylate is located in close proximity of the side chain of Lys192 and might thus be engaged in a favorable electrostatic interaction, although the end of this side chain is quite disordered in most X-ray crystal structures of complexes between F.VIIa and inhibitors. Notably a 'flipped' conformation of the peptide bond between Lys192 and Gly193 is observed (see Fig. 1A).

From examination of the X-ray structure it became obvious that the carboxylate could also be attached directly to the central benzylic position (C_α of the central phenylglycine moiety) and still contribute to the binding affinity by an electrostatic interaction with the quite flexible lysine side chain.

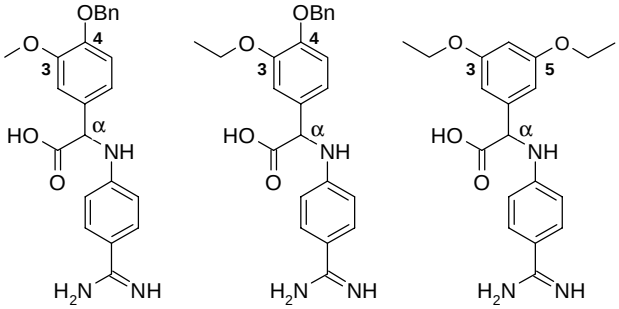
This hypothesis was confirmed by the preparation of the phenylglycine derivative **4** (Table 2). The replacement of the phenylglycine amide side chain by a simple carboxylate still resulted in a binding affinity of 61 nM. However, the selectivity against thrombin was lost. Activity could be improved by a factor of 2 by replacement of the methoxy group in the 3 position by an ethoxy group (compound **5**).

Much better selectivity against thrombin was observed for compounds with two *meta* substituents in both positions 3 and 5 as exemplified by compounds **6** and **7**–**20** (Table 3). This observation can be explained by the unique positioning and shape of the small S2 pocket of F.VIIa. In all X-ray structures of complexes between F.VIIa and phenylglycine as well as phenylglycine amide inhibitors (unpublished results), the S2 pocket is occupied by a small substituent in the position *meta* to C_α of the phenylglycine moiety (Fig. 2B). In thrombin, the S2 pocket is lined by an insertion loop made up of Tyr60A-Pro60B-Pro60C-Trp60D. It is located in a slightly different region of the active site and looks completely different from the S2 pocket of F.VIIa (Fig. 2C).

In the case of F.VIIa, compounds **1**–**20** most likely accommodate their respective 3-substituents in the S2 pocket. In the active site of thrombin, the 3,4-dialkoxy-substituted phenyl residue of compounds **4** and **5** can be rotated by 180° around the bond between C_α and the phenyl glycine phenyl ring, in such a way that the alkoxy group in position 3 is turned out of the active site and thus points toward the solvent. This explains the good inhibitory activity of these two derivatives toward thrombin. In the case of the 3,5-disubstituted phenylglycine derivatives **6**–**20**, the accommodation of the 3,5-dialkoxyphenyl residue in the active site of thrombin by means of such a rotation is no longer possible because two *meta* substituents are present, thus explaining their selectivity against thrombin and F.Xa which lacks the small S2 pocket completely.

There is not much room for optimization of the *meta* substituent in position 3, occupying the small S2 pocket. Besides the ethoxy group, methoxy, methyl, ethyl, vinyl,

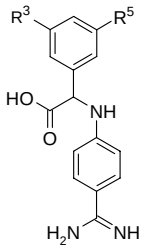
Table 2. Inhibition of TF/F.VIIa and related serine proteases by phenylglycine derivatives **4**–**6**

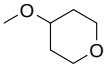
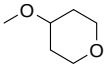
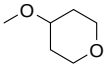
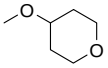
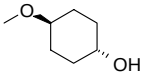
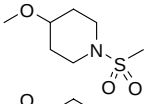
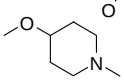


4 **5** **6**

Compound	K_i (μM)				Thrombin	$2 \times PT^a$ (μM)
	F.VIIa	Thrombin	F.Xa	Trypsin	F.VIIa	
4	0.061	0.074	0.73	0.31	1.2	11.9
5	0.028	0.085	0.79	1.0	3.0	8.8
6	0.19	>35	5.6	16.9	184	9.1

Table 3. Inhibition of TF/F.VIIa by *meta*,*meta*-disubstituted derivatives **7**–**20**



Compound	R^3	R^5	K_i (μM)				Thrombin	2 × PT (μM)	2 × aPTT[a] (μM)	aPTT/PT
			F.VIIa	Thrombin	F.Xa	Trypsin	F.VIIa			
7	EtO		0.14	>6.6	2.9	7.0	49.1	4.8	19.8	4.1
8	Ethyl		0.060	1.6	>7.5	4.8	27.2	4.8	15.6	3.3
9	Vinyl		0.069	2.9	13.3	8.7	41.4	5.9	23.7	4.0
10			0.038	4.7	2.9	1.9	123.7	3.4	14.3	4.2
11	EtO		0.11	5.5	12.2	4.0	50.1	5.4	29.4	5.4
12	EtO		0.18	>3.5	4.4	3.5	20.1	4.8	26.9	5.6
13	EtO		0.48	9.9	1.6	8.6	20.1	4.6	22.3	4.8
14	EtO		0.33	8.6	2.3	7.3	25.8	4.5	17.2	3.8
15			0.065	9.5	2.6	5.4	211.8	4.3	12.9	3.0
16	EtO		0.43	11.3	9.1	9.9	26.2	8.6	51.7	6.0
17			0.129	8.8	15.3	6.6	68.1	9.1	34.0	3.7
18	EtO		0.12	4.6	1.5	4.5	40.2	2.7	10.3	3.8
R enantiomer at C_α										
19	EtO		0.13	7.3	1.4	>68	57.1	35.6	69.9	2.0
20	EtO		0.51	10.2	2.2	8.0	20.2	11.8	57.6	4.9

[a] Human citrated plasma is spiked with at least six concentrations of the inhibitor. Clotting via the intrinsic pathway is initiated by the addition of Actin® FS (ellagic acid in soy phosphatides). Clotting time is determined by a turbidity measurement. The concentration of inhibitor necessary to double control clotting time (EC_{50}) is determined by fitting the data to an exponential regression.[11]

ethinyl, propyl, cyclopropyl, and 2-propenyl are allowed in this position as demonstrated by comparing a series of compounds with a tetrahydropyranyloxy substituent in position 5 (Table 3, entries **7**–**10**, and unpublished results). Regarding in vitro and plasma potency (2 × PT) the best compound out of this series is the ethinyl derivative **10**. Additionally it is characterized by a good selectivity against thrombin and F.Xa. Consequently, the intrinsic pathway is inhibited to a much lesser extent than the extrinsic pathway as can be seen by the quite favorable aPTT/PT ratio. The aPTT (2 × prolongation of activated partial thromboplastin time) serves as a measure for the ability of the inhibitor to interfere with the coagulation cascade via the intrinsic pathway.[11] Larger moieties in position 3 lead to a sharp drop in affinity (unpublished data). According to modeling and

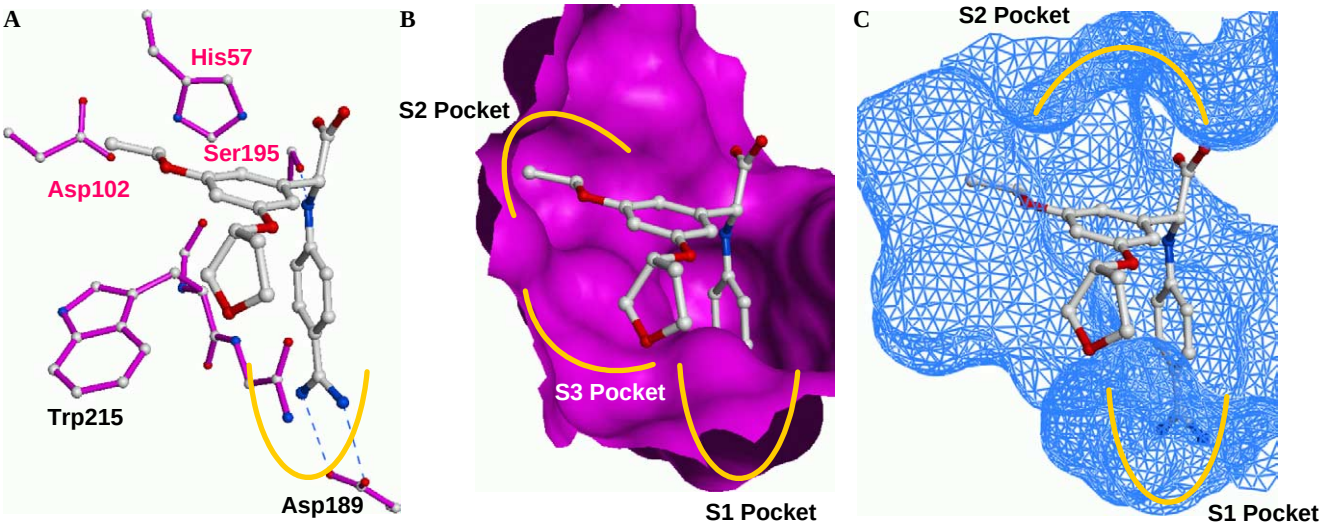


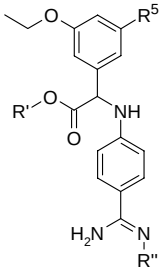
Figure 2. (A) Compound **18** docked in the active site of F.VIIa (magenta: relevant amino acid residues of enzyme). (B) The same representation with the surface of the active site of F.VIIa shown in magenta. (C) Superposition of compound **18** in the F.VIIa binding conformation with the active site of thrombin. The *meta* ethoxy group cannot be accommodated as in F.VIIa because the S2 pocket is different in thrombin.

X-ray structural analysis of selected derivatives (unpublished data), the *meta* substituent in position 5 is located in the shallow, solvent-exposed S3 pocket allowing the introduction of a large variety of larger substituents. Compounds **11**–**13** all featuring six-membered heterocyclic rings are characterized by good PT potencies. Compound **13** which has the poorest in vitro affinity of these compounds is, surprisingly, the most active compound in plasma. This might be attributed to its additional positive charge which enhances its hydrophilicity.

Going from six-membered to five-membered rings, the tetrahydrofuranyloxy substituent with configuration *S* is found to be optimal (compound **14**), while derivative **16** with an *R*-configured tetrahydrofuranyloxy substituent is much less active in plasma. The same tendency was observed for the two derivatives **15** and **17** bearing an ethinyl substituent in position 3. The better plasma activity of *S*-compounds **14** and **15** might be attributed to a residual F.Xa inhibitory activity. Separation of compound **14** into its two epimers led to the most active derivative found within this series. Compound **18** exhibits a plasma activity of 2.7 μM. In all X-ray structures generated from compounds of the phenylglycine series (unpublished results), it is always the stereoisomer with configuration *R* at C_α of the phenylglycine motive which is found in the active site of F.VIIa. From analogy it can be assumed that compound **18** also is most likely the epimer with configuration *R* at C_α.

Good in vitro affinity was observed for the biaryl derivative **19**. It suffered, however, from poor plasma activity. Replacing the phenyl ring in position 5 by a pyridyl moiety led to a substantial loss in activity, while at the same time the plasma activity was restored (compound **20**). This clearly shows that not only the in vitro affinity,

Table 4. Pharmacokinetic parameters (rat) of parent compounds **6** and **14** as well as of the corresponding prodrugs **21**–**23**



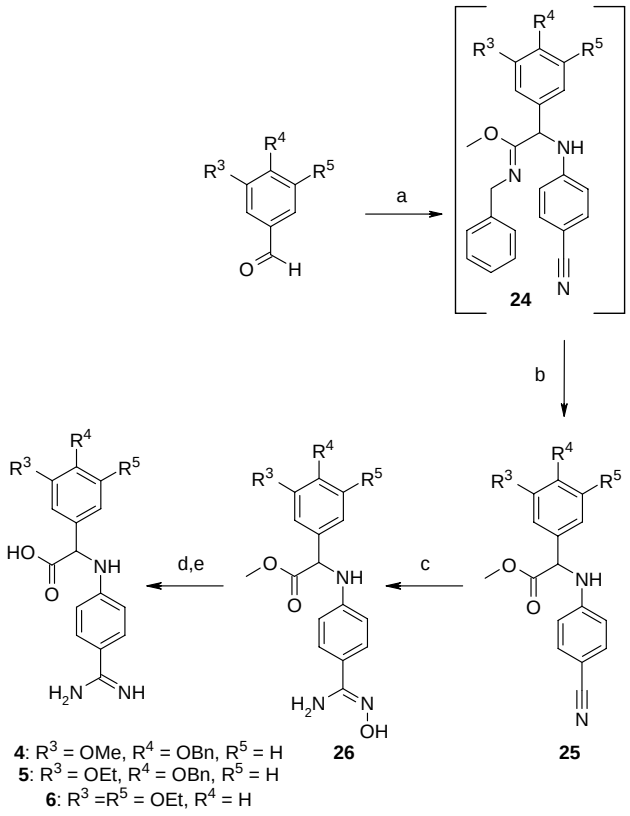
Compound	R^5	R′	R″	Dose (mg/kg)	$T_{1/2}$ iv (h)	$T_{1/2}$ po (h)	Cl (ml/min/kg)	Vdss (l/kg)	*F* (%)
6	EtO	H	H	6.1	2.9	—	2.6	0.3	2
21	EtO	H	OH	10.3	—	5.0	—	—	30
22	EtO	Et	OH	10	3.5	4.8	—	—	100
14		H	H	3	1.4	—	5.7	0.5	—
23		Et	OH	10	0.9	4.1	—	—	15

but to a large extent also the hydrophilicity influences the functional activity of a TF/VIIa inhibitor in plasma. The same effect has been observed by other groups working in the field of thrombin and factor Xa inhibition.[18]

The zwitterionic character of the phenylglycine derivatives renders them very polar. Not surprisingly, the oral bioavailability of **6** amounted to only 2% in the rat (Table 4). When the strongly basic benzamidine group was masked as amidoxime prodrug (compound **21**), the oral bioavailability increased to 30%, while the amidoxime/ester double prodrug **22** displayed an oral bioavailability of 100%. Despite this excellent bioavailability, a very high dose would be required to cover the plasma exposure needed for sufficient PT prolongation because the activity of the parent compound **6** is relatively low ($2 \times PT = 9.1\ \mu M$). Therefore, the PK profiles of the more active tetrahydrofuranyloxy derivative **14** and its amidoxime/ester double prodrug **23** were determined. The bioavailability of **23** in rats was found to be 15%, with a good half-life of about 4 h. Conversion efficiency to parent compound after iv dosing of double prodrug was found to be about 50% for both **22** and **23**. Double prodrug **22** has a bioavailability which is higher than its conversion efficiency observed after intravenous administration of the double prodrug. This indicates very good absorption of the double prodrug and suggests additional conversion of the prodrug during intestinal absorption. In contrast, only partial absorption seems to occur in the case of **23** leading to a bioavailability about 3-fold lower than conversion efficiency. Therefore, dose adjustment and formulation optimization are needed for compound **23** to maintain plasma concentration for sustained inhibition of coagulation activity and are currently being further evaluated.

In summary, we have found novel small molecule factor VIIa inhibitors with good inhibitory activity against factor VIIa and decent in vitro selectivity against other serine proteases of the coagulation cascade and against trypsin. The best derivatives displayed low micromolar functional activity in inhibiting the coagulation cascade via the extrinsic pathway ($2 \times PT$ prolongation), while interfering with the intrinsic pathway to a much lesser extent ($2 \times aPTT$ prolongation). The pharmacokinetic profile of this compound class is characterized by a promising oral bioavailability and a long half-life. Further optimization with the goal to reach sufficient plasma exposure and antithrombotic activity in relevant animal models will be the subject of further publications.

Chemistry. Phenylglycine derivatives **4**–**6** were synthesized starting by a Lewis acid-catalyzed condensation between an appropriately substituted benzaldehyde, 4-aminobenzonitrile, and benzylisonitrile (Scheme 1). The intermediate iminoether **24** was hydrolyzed in situ to the corresponding phenylglycine ester **25** by the addition of an excess of water. By reaction with hydroxylamine hydrochloride compound **25** was converted to the corresponding amidoxime **26**. Reduction with Raney

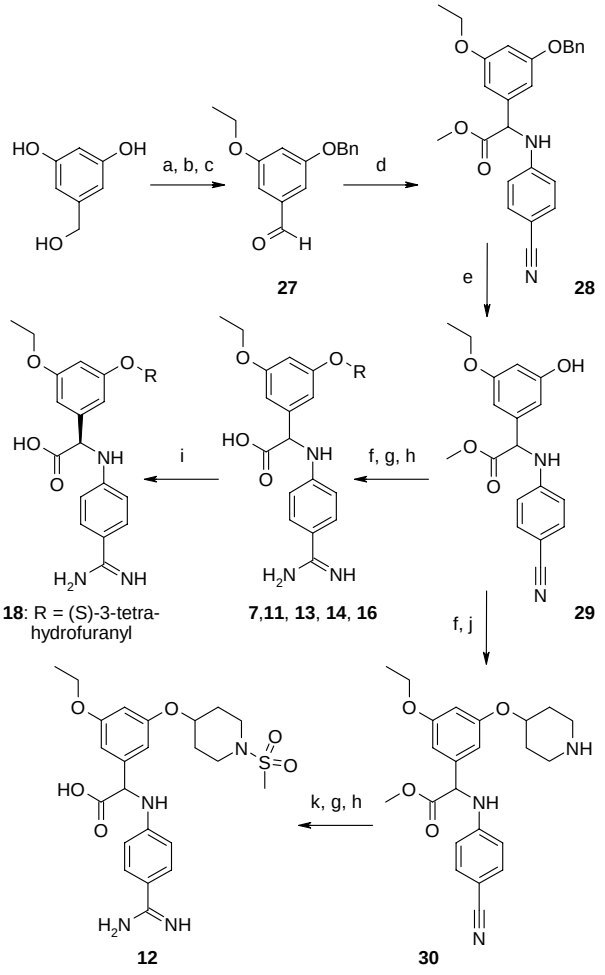


Scheme 1. Preparation of phenylglycine derivatives **4**–**6**. Reagents and conditions: (a) i—4-aminobenzonitrile, MeOH; ii—benzylisonitrile, BF_3-OEt_2, 0 °C; (b) 20 equiv H_2O, 52–80%; (c) H_2N-OH HCl, triethylamine, EtOH, reflux, 66–82%; (d) H_2, Raney nickel, EtOH/H_2O/HOAc, 56–86%; (e) LiOH, THF, 80–98%.

nickel and subsequent ester hydrolysis finally provided the zwitterionic phenylglycines **4**–**6**.

The synthesis of the 3-ethoxy-substituted compounds **7**, **11**–**14**, **16**, and **18** started with the monoethylation of 5-hydroxymethyl-benzene-1,3-diol, followed by a benzylation of the remaining phenol and oxidation of the hydroxymethyl group to give benzaldehyde **27**, which was condensed with 4-aminobenzonitrile and toluene-4-sulfonylisocyanide to furnish phenylglycine ester **28** (Scheme 2). After removal of the benzyl group, phenol **29** was derivatized by a Mitsunobu reaction with various alcohols. Conversion of the nitrile into the respective amidine by a Pinner reaction and hydrolysis of the ester functionality lead to the zwitterionic phenylglycine derivatives **7**, **11**, **13**, **14**, and **16**. The (*S*)-tetrahydrofuranyloxy-substituted derivative **16** was separated into its epimers by chiral HPLC. Compound **12** was prepared starting by derivatization of phenol **29** with 4-hydroxy-piperidine-1-carboxylic acid *tert*-butyl ester. After cleavage of the Boc-protecting group and reaction of the piperidine with methane sulfonylchloride, the nitrile was converted into the amidine by a Pinner reaction. Hydrolysis of the ester led to the desired phenylglycine product **12**.

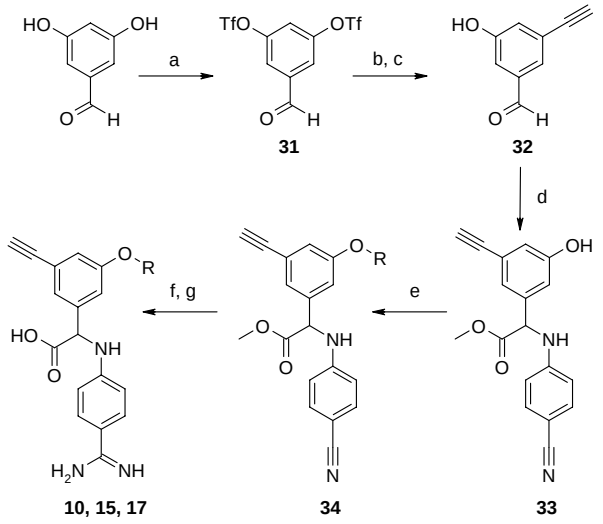
The 3-ethinyl-substituted derivatives **10**, **15**, and **17** were prepared starting with the reaction of 3,5-dihydroxy-benzaldehyde with trifluoromethanesulfonic anhydride



Scheme 2. Preparation of 3-ethoxy-substituted phenylglycine derivatives **7**, **11**–**14**, **16**, and **18**. Reagents and conditions: (a) ethylbromide, K_2CO_3, DMF, 60 °C, 30%; (b) benzylbromide, K_2CO_3, DMF, 65 °C, 83%; (c) MnO_2, 1,2-dichloroethane, 50 °C, 81%; (d) i—4-aminobenzonitrile, MeOH; ii—toluene-4-sulfonylisocyanide, BF_3-OEt_2, 0 °C; iii—20 equiv H_2O, 63%; (e) H_2, Pd/C, THF, EtOH, 70%; (f) R″-OH, PPh_3, DEAD, THF, 63–68%; **7** and **13**: R″ = R′ = R; **14** and **16**: R′ = R; **11**: R″-OH = *cis*-4-trityloxy-cyclohexanol; **14**: R″-OH = (*R*)-3-hydroxy-tetrahydrofurane; **16**: R″-OH = (*S*)-3-hydroxy-tetrahydrofurane; **30**: R″-OH = 4-hydroxy-piperidine-1-carboxylic acid *tert*-butylester (Derivative **11**: The trityl group of R″-OH is lost during the Pinner reaction. Derivatives **11**, **14**, **16**: Mitsunobu reaction with the respective alcohols R″-OH proceeds with inversion of stereochemistry.) (g) HCl-gas, $CHCl_3$–MeOH (3:1), −10 °C, evaporate, then 2 N NH_3 in MeOH, 45–92%; (h) LiOH, THF, 62–97%; (i) separation of **16** by chiral HPLC, 31%; (j) 4 N HCl in EtOAc, 94%; (k) CH_3-SO_2-Cl, DIPEA, THF, 44%.

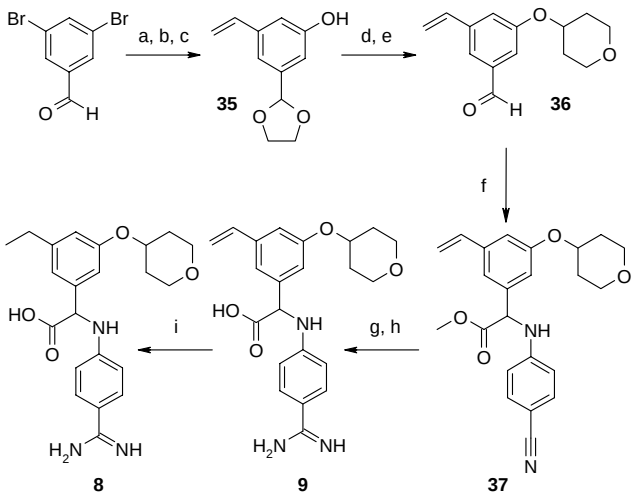
(Scheme 3). Mono-Sonogashira coupling and cleavage of the remaining triflate lead to intermediate **32** which was condensed in a Lewis acid-catalyzed three-component reaction with 4-aminobenzonitrile and toluene-4-sulfonylisocyanide to give phenylglycine ester **33**. The phenol was derivatized with various alcohols via a Mitsunobu reaction. Finally, the nitrile was converted into the corresponding amidine by a Pinner reaction. The final products **10**, **15**, and **17** were generated by hydrolysis of the corresponding phenylglycine esters.

The vinyl-substituted phenylglycine derivative **8** and the corresponding ethyl compound **9** were obtained



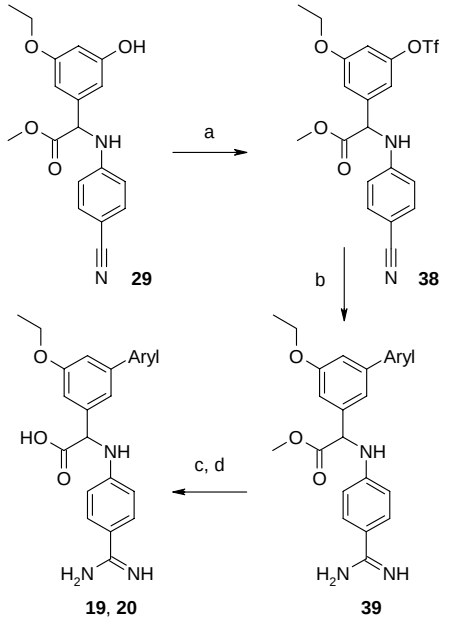
Scheme 3. Preparation of 3-ethinyl-substituted derivatives **10**, **15**, and **17**. Reagents and conditions: (a) trifluoromethanesulfonic anhydride, TEA, CH_2Cl_2, 0 °C, 88%; (b) trimethylsilylacetylene, $Pd(PPh_3)_2Cl_2$, Cu(I)I, TEA, THF, 70%; (c) 1 N LiOH, MeOH, 66%; (d) i—4-aminobenzonitrile, MeOH; ii—toluene-4-sulfonylisocyanide, BF_3-OEt_2, 0 °C; iii—20 equiv H_2O, 65%; (e) R′-OH, PPh_3, DEAD, THF, 62–85%; **10**: R = R′; **15**: R′-OH = (*R*)-3-hydroxy-tetrahydrofurane; **17**: R′-OH = (*S*)-3-hydroxy-tetrahydrofurane (Derivatives **15** and **17**: Mitsunobu reaction with the respective alcohols R′-OH proceeds with inversion of stereochemistry.) (f) HCl-gas, $CHCl_3$–MeOH (3:1), −10 °C, evaporate, then 2 N NH_3 in MeOH, 41–50%; (g) LiOH, THF, 60–88%.

starting by ethylene glycol protection of 3,5-dibromobenzaldehyde, followed by a mono-Stille coupling with tributyl(vinyl)tin and subsequent conversion of the remaining bromo substituent into a hydroxy group by

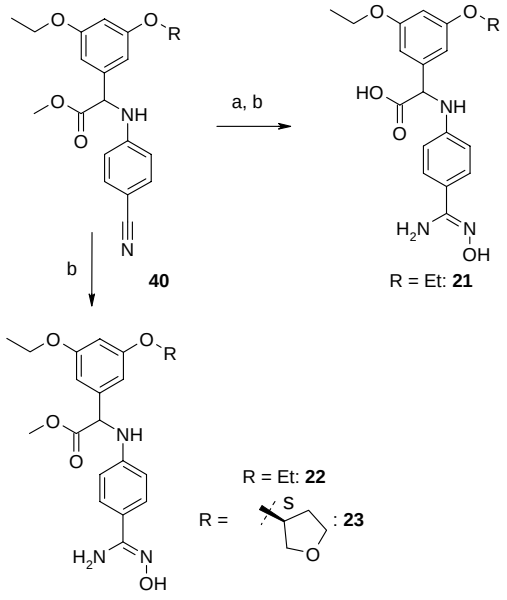


Scheme 4. Preparation of 3-ethyl-substituted derivative **8** and 3-vinyl-substituted **9**. Reagents and conditions: (a) *p*-TsOH, ethylene glycol, benzene, reflux, 98%; (b) tributyl(vinyl)tin, tetrakis(triphenylphosphin)palladium(0), toluene, 80 °C, 64%; (c) *n*-butyllithium, trimethylborate, THF, −78 °C, then acetic acid, H_2O_2, 68%; (d) 4-hydroxy-tetrahydropyrane, PPh_3, DEAD, THF, 69%; (e) 2 N HCl, THF, 60%; (f) i—4-aminobenzonitrile, MeOH; ii—toluene-4-sulfonylisocyanide, BF_3-OEt_2, 0 °C; iii—20 equiv H_2O, 66%; (g) HCl-gas, $CHCl_3$–MeOH (3:1), −10 °C; evaporate, then 2 N NH_3 in MeOH, 86%; (h) LiOH, THF, 74%; (i) H_2, Pd/C, EtOH/H_2O, 66%.

lithiation, reaction with trimethylborate, and subsequent oxidation with hydrogen peroxide (Scheme 4). Intermediate **35** was reacted in a Mitsunobu reaction with 4-hydroxy-tetrahydropyrane and then deprotected. The resulting aldehyde **36** was converted in a Lewis acid-catalyzed condensation with 4-aminobenzonitrile and toluene-4-sulfonylisocyanide to provide phenylglycine ester **37**. Conversion of the nitrile into an amidine group and hydrolysis of the ester moiety led to the phenylglycine compound **8** which was converted into the ethyl analogue **9** by hydrogenation of the double bond.



Scheme 5. Preparation of biaryl derivatives **19** and **20**. Reagents and conditions: (a) trifluoromethanesulfonic anhydride, TEA, dichloromethane, 0 °C, 99%; (b) **19**: phenylboronic acid, K_2CO_3, tetrakis(triphenylphosphine)palladium(0), toluene, 90 °C, 93%; **20**: 3-(tri-*n*-butylstannyl)-pyridine, triphenylarsine, $Pd_2(dba)_3$-$CHCl_3$, LiCl, NMP, 80 °C, 60%; (c) HCl-gas, $CHCl_3$–MeOH (3:1), −10 °C, evaporate, then 2 N NH_3 in MeOH, 77–87%; (d) LiOH, THF, 90–94%.



Scheme 6. Preparation of prodrugs **21**–**23**. Reagents and conditions: (a) LiOH, THF, 86%; (b) H_2N-OH hydrochloride, TEA, EtOH, reflux, **21**: 22%, **22**: 81%, **23**: 65%.

The 5-aryl-substituted compounds **19** and **20** were synthesized starting from phenol **29** (Scheme 2) which after conversion to triflate **38** was reacted with phenylboronic acid and 3-(tri-*n*-butylstanyl)-pyridine (Scheme 5). Conversion of the nitrile into the amidine via Pinner reaction and hydrolysis of the phenylglycine ester provided the desired products.

The amidoxime prodrug **21** was synthesized starting from the 3,5-diethoxy-substituted phenylglycine ester **40** (prepared as described in Scheme 1) by hydrolysis of the ester group and subsequent reaction of the nitrile with hydroxylamine hydrochloride (Scheme 6). The amidoxime/ester double prodrugs **22** and **23** were prepared by reaction of the appropriately substituted phenylglycine esters **40** (prepared as described in Schemes 1 and 2) with hydroxylamine hydrochloride.

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