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Factor VIIa inhibitors: Improved pharmacokinetic parameters

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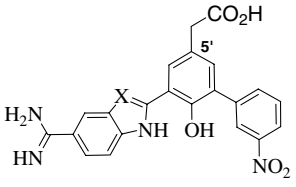
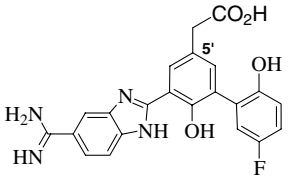
Abstract—Efforts to improve the potency and pharmacokinetic properties of small molecule factor VIIa inhibitors are described. Small structural modifications to existing leads allow the modulation of half-life and clearance, potentially making these compounds suitable candidates for drug development.

We have previously identified small molecule serine protease inhibitors of which analogs **1**–**3** are exemplary of this series.[1,2] As described earlier[2–4] our goal was to develop potent and selective small molecule inhibitors of the factor VIIa/Tissue Factor (fVIIa/TF) complex for the treatment of thromboembolic disease. We were particularly interested in achieving >1000-fold selectivity for the fVIIa/TF complex versus factor Xa (fXa) and thrombin as it has been postulated that such selectivity may be important for improving the safety profile of anticoagulants.[5] Additionally, we were interested in generating analogs that would be amenable to once daily subcutaneous dosing in human.



1: X = N; **2**: X = CH



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The early leads **1** and **2** lacked the desired selectivity against fXa and contained an arylnitro-group, which has the potential to form toxic metabolites. In response to these concerns, the nitrophenyl group was replaced with a 2″-hydroxy-5″-fluorophenyl group, as in **3**, which led to a dramatic increase in selectivity for fVIIa over fXa as shown in Table 1.[3] Compounds **1**–**3**, however, were cleared rapidly following intravenous (iv) administration to rats, and the MRT was less than 1 h.[6] It was found that glucuronidation is the major mode of metabolism.[7] In the present paper, we describe our efforts to improve the potency, selectivity, and pharmacokinetics of our series to achieve analogs that would be suitable for further development.

X-ray crystallography data indicate that the acetic acid moiety off the 5′ position in structures **1**–**3** forms a favorable interaction with the Lys192 of fVIIa.[1,2] The same carboxylate anion repels Glu192 of thrombin contributing to the enhanced selectivity against thrombin. As Lys60A of fVIIa is also near this region, we decided to build in another acidic moiety here to take advantage of this potential interaction. Additionally, this 5′-vector also accesses solvent and could simultaneously be used as a handle to manipulate physiochemical properties which in turn may alter the pharmacokinetic parameters. We were

Table 1. SAR and pharmacokinetic parameters of early analogs in rat following IV administration

Compound	fVIIa/TF K_i (μM)	Selectivity		CL[a] (mL/min/kg)	MRT[b] (h)
		fXa	fIIa		
1	0.015	20	>10,000	9.2	<1
2	0.003	20	960	10.5	<1
3	0.002	2600	>75,000	8.1	<1

[a] CL, plasma clearance.
[b] MRT, mean residence time.

Keywords: Factor VIIa; Tissue factor; Serine protease inhibitor; Anticoagulants; Amidines; Dicarboxylic acids.
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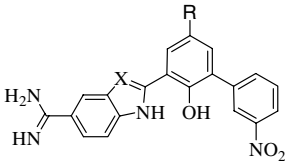
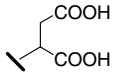
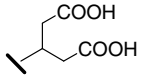
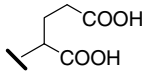
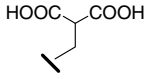
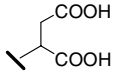
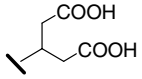
Table 2. Structure–activity relationship and pharmacokinetic parameters of diacid analogs (3″-nitrophenyl series) in rat following IV administration



R	Compound	X	fVIIa/TF K_i (μM)	Selectivity		CL[b] (mL/min/kg)	MRT[c] (h)
				fXa	fIIa		
	**4**[a]	N	0.022	40	9545	0.04	>24
	**5**	N	0.036	25	5000	na	na
	**6**[a]	N	0.017	40	9000	0.5	5
	**7**	N	0.021	20	40,000	5.2	<1
	**8**[a]	CH	0.001	50	700	0.1	>24
	**9**	CH	0.013	5	3900	1.8	1.5

[a] Racemic mixture.
[b] CL, plasma clearance.
[c] MRT, mean residence time.

looking to identify compounds that did not suffer from extensive glucuronidation and offered a good pharmacokinetic profile to support once daily dosing in human. Toward this goal, a series of diacids were generated off the 5′ position of scaffolds **1** and **2**, as depicted in Table 2. When we began this work, the more selective and preferred 2″-hydroxo-5″-phenyl analog **3** had not yet been uncovered so the work was initiated in the arylnitro series. We generated analogs in both the benzimidazole and indole series in order to evaluate the SAR in parallel. Succinic (**4** and **8**), 3-pentanedioic (**5** and **9**), 2-pentanedioic (**6**), and methylmalonic (**7**) acids were chosen to explore the region near the Lys60A and Lys192. The benzimidazole analogs (**4**–**7**) ranged in potency from 17 to 36 nM, all showing only a slight change in binding energy as compared to compound **1**. Indole derivatives (**8**–**9**), although more potent, show a similar trend in SAR. Selectivity against fXa remains low in both benzimidazole and indole series.

Although we did not realize any gains in potency in analogs **4**–**9** as compared to **1** and **2**, we did achieve some dramatic alterations in pharmacokinetic properties (Table 2). While monocarboxylic acids **1** and **2** demonstrate relatively high CL, introduction of a succinic acid moiety directly bound to the central phenol ring dramatically decreases CL and increases MRT (Fig. 1). This improvement in pharmacokinetics seems to be directly related to the succinic acid. In vivo half-life substantially decreases when two carboxylate groups become separated by one more methylene group (viz. **6**, **9**). The pharmacokinetic properties of these pentanedioic acids depend on their structure—the asymmetrical one being more stable. It should be noted that compounds with asymmetrical diacids were tested as a racemic mixture of the enantiomers. These are likely to undergo metabolic transformation at different rates, so the pharmacokinetic parameters represent a statistical average of an ever-changing ratio. Metabolism of the malonic acid derivative **7** may in part involve a mechanism other than glucuronidation (decarboxylation) resulting in a faster clearance.

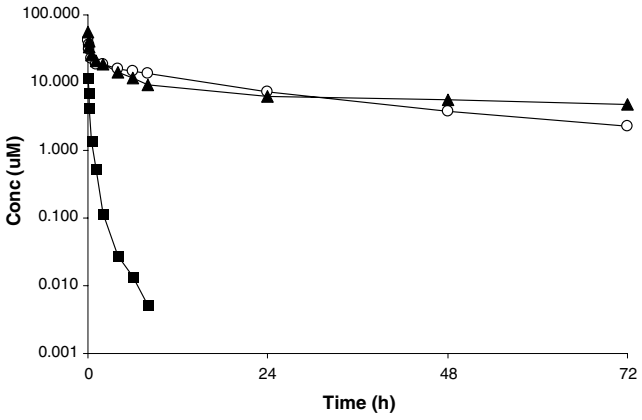
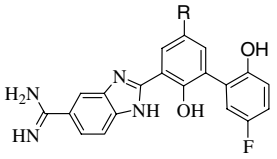
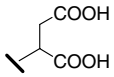
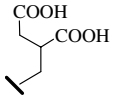


Figure 1. Concentration versus time profile of analogs **2** (■), **4** (▲), and **8** (○) in rat following IV administration at 1 mg/kg.

Table 3. Structure–activity relationship and pharmacokinetic parameters of diacid analogs (2″-hydroxy-5″-fluorophenyl series) in rat following IV administration



R	Compound	fVIIa/TF K_i (μM)	Selectivity		CL[b] (mL/min/kg)	MRT[c] (h)
			fXa	fIIa		
	**10**[a]	0.004	1800	55,500	0.3	9
	**11**[a]	0.004	1250	37,000	2.6	<1

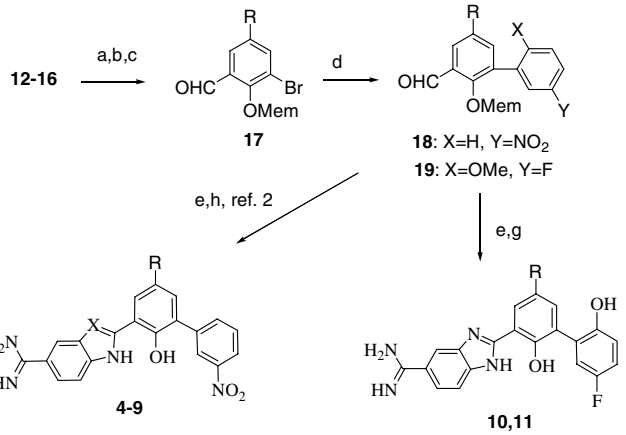
[a] Racemic mixture.
[b] CL, plasma clearance.
[c] MRT, mean residence time.

Our next goal was to transfer the 'PK SAR' that we learned on scaffolds **1** and **2** to our more selective series **3**. Toward this end, we placed a succinic acid moiety at the 5′-position giving analog **10**. Similar to the aryl nitro series, no potency improvement was obtained with this modification, but the pharmacokinetic enhancement did transfer (Table 3), resulting in identification of phenylsuccinic acid derivative **10** with not only excellent potency and selectivity but also a good pharmacokinetic profile in rat. Again, deviation from phenylsuccinic acid moiety (separation by one methylene group, as in **11**) resulted in a sharp decrease in stability in vivo.

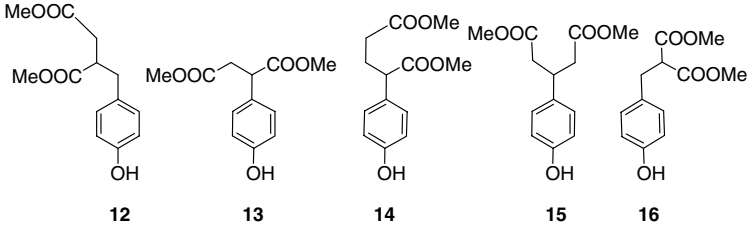
Oral bioavailability evaluations in rat, with selected analogs in this series, demonstrated limited absorption (i.e., <1%), most likely due to the charged succinate and amidino groups. Some analogs (e.g., **10**) show high bioavailability (i.e., >90%) after subcutaneous administration in rats. Combined with their good pharmacokinetic profiles, these compounds represent promising candidates as parenteral anticoagulants.

Compounds **4**–**11** were obtained by modifying known synthetic procedures[2,3] as depicted in Scheme 1. The necessary 4-substituted phenol intermediates **12**–**16** were each prepared in a unique manner. Methyl ether and alkyl ester groups (where applicable) were removed by heating the corresponding anisoles in aqueous hydrogen bromide yielding phenolic derivatives of dicarboxylic acids, which were then reesterified.

Reaction of 4-iodoanisole and dimethylitaconate using Heck reaction conditions[8] and subsequent hydrogenation led to 2-(4-methoxy-benzyl)-succinic acid dimethyl ester (precursor to **12**). The same method was applied for the preparation of **13**, although 4-iodophenol and diethylfumarate were used as the starting materials.[3] Dimethyl ester of 2-(4-methoxy-phenyl)-pentanedioic acid (precursor to **14**) was synthesized by addition of methylacrylate to 4-methoxyphenylacetate.[9] Dimethyl



Scheme 1. Reagents and conditions: (a) HCHO, $MgCl_2$, triethylamine, MeCN, heat; (b) NBS, DMF; (c) MEM-Cl, Hunig's base, DCM; (d) Arylboronic acid, $Pd(PPh_3)_4$, 2 M aq Na_2CO_3, dimethoxyethane, reflux; (e) 3,4-diaminobenzamidine, benzoquinone, MeOH, reflux; (h) 4 M HCl aq, 100 °C; (g) 48% HBr aq, 100 °C.



ester of 3-(4-methoxy-phenyl)-pentanedioic acid (precursor to **15**) was obtained by reaction of 4-methoxybenzaldehyde with ethyl acetoacetate catalyzed by piperidine followed by alkali hydrolysis.[10] Commercially available 2-(4-hydroxy-benzylidene)-malonic acid dimethyl ester was directly hydrogenated to malonic ester **16**. Phenol derivatives **12**–**16** were then transformed into the final benzimidazole and indole targets **4**–**11** via a series of well-precedented steps. Formylation of each phenol **12**–**16** with paraformaldehyde and magnesium chloride, bromination with NBS, and subsequent MEM-protection of the phenol group afforded compounds of general formula **17**. Each aldehyde, as in **17**, was then subjected to coupling with commercially available boronic acids under Suzuki reaction conditions producing compounds of the general structure **18** and **19**. Corresponding benzimidazoles **4**–**7** and **10**, **11** were synthesized by an oxidative cyclization of diaminobenzamidine onto the aldehyde group of **18** followed by cleavage of the protecting groups. Synthesis of the indoles **8**, **9** was conducted using Sonogashira coupling route starting from aldehydes **19** as previously described.[2]

In summary, a series of potent and selective dicarboxylic acid analogs were generated as factor VIIa/TF inhibitors. The pharmacokinetic properties of these analogs after IV dosing to rat were dramatically different. The succinic acid analog **10** shows the best pharmacokinetic profile and will be the focus of future lead optimization for development of a parenteral anticoagulant.

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6. Plasma concentrations of the compounds were determined by LC/MS/MS. The plasma sample was processed using acetonitrile precipitation and then the supernatant was injected onto the LC column. The limit of quantitation of the assay was 1–6 nM. Pharmacokinetic data were analyzed by WinNonlin-Pro (Pharsight Corp.), using compartmental analysis. Pharmacokinetic parameters, including the area-under-the-curve (AUC), clearance (CL), volume of distribution (V_d), and mean residence time (MRT), were determined. Clearance and mean residence time were calculated as follows: CL = Dose/AUC and MRT = V_d/CL.
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