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Efforts toward oral bioavailability in factor VIIa inhibitors

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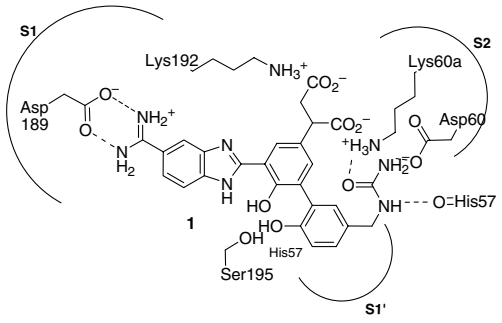
Abstract—Efforts toward developing orally bioavailable factor VIIa inhibitors starting from parenteral lead compound **1** are described. SAR resulted in improved physicochemical properties, leading to enhanced oral absorption in rat.

There is increasing interest in developing oral anti-coagulants with improved therapeutic efficacy over the existing standard of care.[1] Our approach toward developing an anticoagulant is the direct inhibition of factor VIIa (fVIIa), a key enzyme in the coagulation cascade.[2,3] In this communication, we disclose our efforts to improve the oral bioavailability of our lead fVIIa inhibitors by modifying its physicochemical properties.

We have reported the development of potent and selective small-molecule inhibitors of the factor VIIa/tissue-factor (fVIIa/TF) complex.[3] An example, compound **1**, is efficacious after bolus intravenous (iv) dosing in a baboon model of arterial thrombosis.[3] Expectedly, compounds similar to **1** with amidine and diacid functionalities had limited oral absorption when dosed in rats. We recognized the need to modify the properties of this class of compounds to lead us toward oral bioavailability.[4] Of concern was the charged nature of the amidine and diacid functionalities. Combined with the urea, these lead to a high polar surface area (PSA) and molecular weight (MW).

We undertook an analysis of the role of each part of compound **1** in the potency and selectivity profile, as outlined in Figure 1a. This analysis is based on a crystal structure of **1** in fVIIa.[3] The amidine interacts with

a



b

fVIIa[5] K_i(μM)	2xPT[6] (μM)	fVIIa Selectivity against Thrombin	fXa	Trypsin	PSA ($^oA^2$)	MW
0.001	1.9	100,000	3,600	10,000	248	532

Figure 1. (a) Representation of key interactions of **1** with fVIIa. (b) In vitro profile and calculated physicochemical parameters for compound **1**.

Asp189 in the S1 pocket. A prodruging approach (as the hydroxy amidine) would be employed to neutralize the charge and help improve absorption. The success of this approach has been demonstrated in the development of the thrombin inhibitor Ximelagatran.[7] The amidine, in conjunction with the imidazole NH and the 2-phenolic OH, is integral to the binding of this class of inhibitors to trypsin-like enzymes.[8] In addition, the distal 2′-phenol offers further fVIIa potency and

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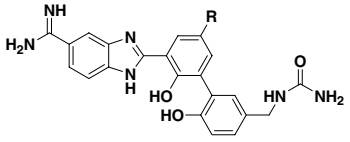
selectivity against other trypsin-like enzymes.[9] Therefore, our plan was to preserve these interactions, while exploring the possibility of replacing the succinic acid and urea substituents. The goal was to considerably reduce the MW and PSA.

The succinic acid at the 5-position improves fVIIa potency by interacting with Lys192, while enhancing thrombin selectivity through negative interactions with Glu192. In our earliest reports of fVIIa inhibitors, the 5′-acidic group played a very important role in potency and selectivity gains.[2a] More importantly, we have utilized this position as a handle to modulate pharmacokinetic properties.[10] With the discovery of the distal urea functionality,[3] we reasoned that the 5-acidic group may be expendable. Numerous 5-substituted analogs were synthesized and evaluated, of which, selected examples are shown in Table 1. We noted that replacing the succinic acid with small non-polar substituents such as $-CH_3$, –F, and –H had minimal effect on the in vitro parameters.[5] The fVIIa potency was unaffected by these changes and the selectivity profile remained impressive. Indeed, the crystal structure of **1** in fVIIa had revealed numerous other points of interaction with the enzyme, which are most likely maintained in this new set of compounds. It was gratifying that the in vitro efficacy, as measured by the $2 \times PT$[6] assay, did not change. However, as suspected, the pharmacokinetic profile of the newly synthesized analogs suffered from rapid clearance. After iv dosing in rat, compound **1** has a mean residence time (MRT) of greater than 1 h,[3] while the analogs **2**–**5** had MRTs of less than 20 min (data not shown). Therefore, while removing the succinic acid from compound **1** allowed us to considerably reduce the PSA and molecular weight, we were left with the need for further optimization.

Next, we examined the role of the urea functionality on the distal aryl ring. As depicted in Figure 1a, it makes specific contacts with His57, Asp60, and Lys60a. Among numerous analogs made, we found that amides which lost the interaction with Asp60 but preserved the 'CH_2–NHC(O)' part of the urea were reasonably well tolerated and retained desirable in vitro characteristics. Selected examples (**6**–**10**) of analogs replacing the urea are shown in Table 2.

These include alkyl amides (**7** and **10**), amides incorporating polar groups (**6** and **8**) as well as a disubstituted urea **9**. These analogs were prepared with an indole replacing the benzimidazole ring. Based on previous experience with this class of compounds,[2] we were confident that this change would be well tolerated, while providing another opportunity to lower the PSA. The loss of the Asp60 interaction in the amide series is reflected in an overall loss of selectivity, particularly

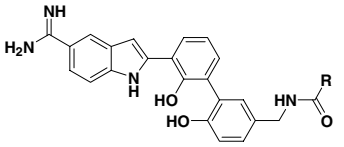
Table 1. SAR at the 5′-position



Compound	R	fVIIa K_i[5] (μM)	$2 \times PT$[6] (μM)	Selectivity against			PSA[a] (°A^2)	MW
				Thrombin	fXa	Trypsin		
1	$CH(COOH)CH_2COOH$	0.001	1.9	100,000	3600	11,333	250	532
2	CH_2COOH	0.014	—	10,700	235	328	213	475
3	CH_3	0.004	2.0	10,900	303	584	175	431
4	F	0.005	2.5	11,700	404	744	175	435
5	H	0.014	2.3	6923	207	276	175	417

[a] Polar surface area.

Table 2. SAR to replace the urea



Compound	R	fVIIa K_i (μM)	$2 \times PT$ (μM)	Selectivity against			PSA (°A^2)	MRT[a] (min)
				Thrombin	fXa	Trypsin		
6	CH_2OH	0.003	0.9	1233	60	400	157	117
7	CH_3	0.12	—	1250	6	142	135	—
8	$CH_2CH_2CH_2N(Me)_2$	0.016	0.9	3375	37	181	138	50
9		0.017	2.0	3117	28	194	147	—
10	$CH_2(CH_2)_3CH_3$	0.013	1.4	7222	107	237	135	89

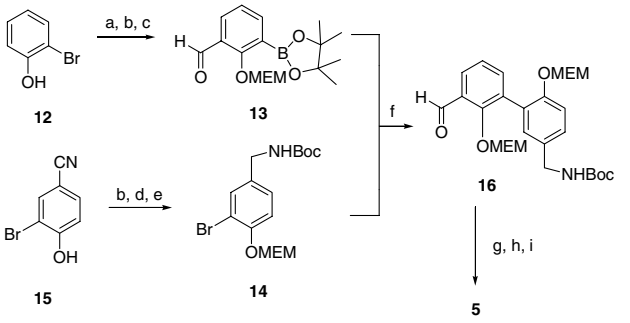
[a] Mean residence time.

against fXa. Selected compounds were dosed iv (intravenous) to male Sprague–Dawley rats and the pharmacokinetic parameters compared. The MRTs are listed in Table 2. Our SAR leads us to the hexanoyl amide analog **10** which had the best combination of lowered PSA and MW as well as in vitro potency, efficacy, and selectivity. In addition, this lead offered pharmacokinetic parameters which warranted further evaluation. Since we were aware, based on our previous study,[11] that even simple amidino compounds (without distal ring substituents) in this series had poor oral absorption, we decided to convert amidino group in **10** to a neutral hydroxyamidine in order to improve chances of oral absorption.

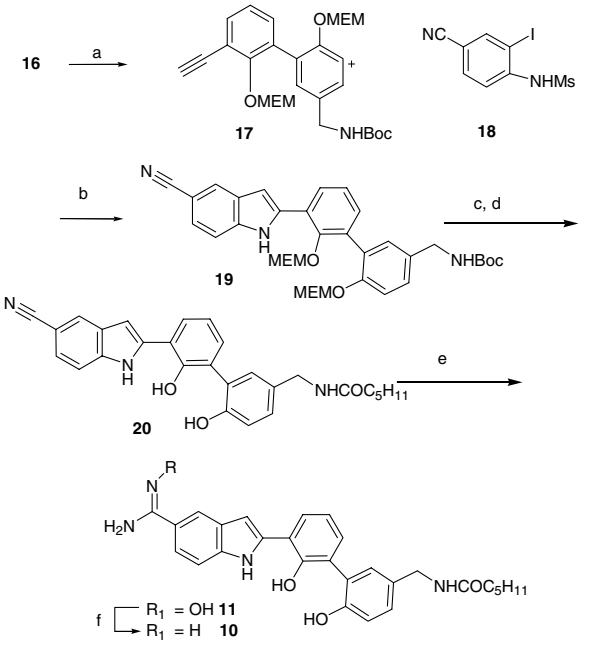
Compound **11**, the hydroxyamidine prodrug of **10**, was dosed orally at 10 mg/kg to male Sprague–Dawley rats with jugular vein and portal vein cannulae.[12] Monitoring the blood levels of both prodrug **11** and the parent compound **10** revealed that while the oral bioavailability of **11** was 11%, that of the parent was negligible[13] (Table 3).

Scheme 1 outlines the synthesis of benzimidazole compound **5**. The commercially available bromo phenol **12** was selectively ortho formylated with paraformaldehyde and magnesium chloride as reported previously.[14] The bromo salicylaldehyde intermediate thus obtained was protected as its MEM ether and the aryl bromide converted to the pinacol boronate ester **13** using bis-(pinacolato)diboron with $PdCl_2$(dppf) as a catalyst.[15] Aryl bromide **14** was obtained from **15** in three steps via phenol protection as the MEM ether, reduction of the nitrile followed by protection of the benzyl amine as its Boc derivative. Suzuki coupling of **13** with aryl bromide **14** furnished the biaryl aldehyde **16**. Oxidative condensation of the aldehyde **16** with 3,4-diamino-benzamidine,[9] removal of protecting groups (MEM ethers and Boc) followed by treatment of resultant benzyl amine with potassium cyanate gave urea **5**. Other benzimidazole derivatives **2**–**4** were prepared in an analogous manner starting from the appropriately substituted salicylaldehyde.

To prepare indole derivative **10** and hydroxy amidine**11** (Scheme 2), aldehyde **16** was converted to alkyne **17** using dimethyl-1-diazo-2-oxopropylphosphonate.[16] Palladium-mediated coupling of the alkyne **17** with *N*-(4-cyano-2-iodo-phenyl)-methanesulfonamide **18**[17] followed by alkaline hydrolysis of the mesylate gave



Scheme 1. Synthesis of amidine **5**. Reagents and conditions: (a) $MgCl_2$, $(HCHO)_n$, NEt_3, CH_3CN, reflux; (b) MEMCl, Hunig's base, DCM, rt; (c) bis-pinacolato diborane, Pd(II), dioxane, reflux, K_2CO_3; (d) diborane, THF reflux; (e) $(Boc)_2$, NEt_3 THF; (f) $Pd(PPh_3)_4$, 2 N K_2CO_3, toluene, reflux; (g) 3,4-diamino-benzamidine, DMF, 1,4-benzoquinone, 60 °C; (h) 4 M HCl in dioxane, rt; (i) KOCN, MeOH rt.



Scheme 2. Synthesis of amidine **10** and hydroxyamidine **11**. Reagents and conditions: (a) (1-diazo-2-oxo-propyl)-phosphonic acid dimethyl ester, K_2CO_3, MeOH, rt; (b) i—$Pd(PPh_3)_2Cl_2$, CuI, NEt_3, THF, 60 °C; ii—50% aq NaOH, MeOH 60 °C; (c) 4 M HCl in dioxane; (d) hexanoyl chloride in DCM and NEt_3, rt; (e) NH_2OH, EtOH, reflux; (f) i—Ac_2O, AcOH, rt; ii—$Pd(OH)_2$ on C, MeOH, atm of H_2.

Table 3. Pharmacokinetics, bioconversion, and oral bioavailability of prodrug **11** following iv and oral administration in rats

PK parameter[12] (po 10 mg/kg, $n = 3$)	Prodrug (**11**)		Parent (**10**)	
	PVC[a]	JVC[b]	PVC[a]	JVC[b]
C_{max} (μM)	1.31	1.73	0.014	0.012
T_{max} (min)	31	30	40	30
AUC (μM min)	122	144	0.88	0.73
Terminal $t_{1/2}$ (min)	49	54	26	37
Oral absorption (%)	9		—	
Oral bioavailability (%)	11		Negligible	

[a] PVC, blood samples collected from portal vein catheter.
[b] JVC, blood samples collected from jugular vein catheter.

triaryl compound **19**. Removal of protecting groups (MEM and Boc) with 4 M HCl in dioxane, followed by treatment of the resulting benzyl amine with hexanoyl chloride, generated amide **20**. Hydroxyamidine prodrug **11** was generated by heating amide **20** with aqueous hydroxylamine in ethanol. To prepare amidine **10**, hydroxyamidine **11** was acylated and reduced under hydrogenation conditions. Other indoles (**6**–**9**) were prepared in an analogous manner.

We have described SAR that has allowed us to evolve from compound **1**, which is suitable for development as a parenteral anticoagulant agent, to lead compound **10**, which may be explored as an orally administered anticoagulant. We demonstrated that it is possible to considerably reduce MW and PSA of **1**, while maintaining suitable in vitro characteristics. The strategy of mitigating the charge of the amidine in the form of a prodrug was successful in improving oral bioavailability of the prodrug. Our studies with the hydroxyamidine prodrug **11** revealed that it is not reduced to the parent **10** in vivo in rat. Efforts directed at exploring other prodrugs which will be cleaved in vivo and offer improved absorption will be the subject of future publications.

Acknowledgments

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