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Factor VIIa inhibitors: A prodrug strategy to improve oral bioavailability

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Abstract—We have developed a series of potent and selective factor VIIa inhibitors based on the 2-[5-(5-carbamimidoyl-1H-benzoimidazol-2-yl)-6-hydroxy-biphenyl-3-yl]-succinic acid scaffold. These amidine-containing compounds have low oral bioavailability. Herein, we describe our efforts to improve the oral bioavailability of the parent amidine via a prodrug strategy where the amidine basicity and polarity were reduced with either an alkoxy-amidine or a carbamate prodrug.

The development of novel therapeutic agents for the treatment of coagulation disorders, such as deep vein thrombosis (DVT) and pulmonary embolism (PE), is a major focus of research in the pharmaceutical industry.[1–3] A number of coagulation proteases have been targeted for the development of an anticoagulant therapy, most commonly thrombin and factor Xa. While inhibition of these enzymes has proven beneficial in the clinic, safety concerns, such as bleeding risk, are problematic. Studies evaluating bleeding tendency[4] and surgical blood loss[5] have shown that inhibition earlier in the coagulation cascade, e.g., factor VIIa, may provide an increased window of safety over inhibition downstream in the cascade with factor Xa and thrombin.[5–7] These possible safety advantages lead us to focus on the development of an oral and selective factor VIIa inhibitor.

We have previously reported on the development of the 2-[5-(5-carbamimidoyl-1H-benzoimidazol-2-yl)-6-hydroxy-biphenyl-3-yl]-succinic acid scaffold as an effective factor VIIa inhibitor.[8,9] The potency of this series is mediated by two principle binding interactions. First, a phenol moiety forms a unique network of hydrogen bonds to the catalytic Ser195, common to all proteases in this family.[10–14] Second, this series utilizes a basic amidine to form a salt bridge with acidic Asp189 in the P1-pocket of the protease. Unfortunately, the amidine is associated with sub-optimal oral bioavailability due to its charged nature ($pK_a \sim 12$). One strategy to increase the oral bioavailability of an amidine is to make a prodrug. Ideally, a prodrug of an amidine would mask its polar basicity, and upon absorption of the prodrug from the gastrointestinal tract into circulation, the prodrug would be cleaved to the active parent amidine. This approach has been successfully demonstrated in the clinic with a number of amidine-containing compounds, most notably ximelagatran, the hydroxy amidine prodrug of melagatran, which is being developed as a direct thrombin inhibitor.[15]

The parent amidine **1** and its associated des-amidine counterpart **2** (Table 1) were administered intravenously (IV) and orally (PO) to male Sprague–Dawley rats ($n = 3$, rats/compound per route) to evaluate the oral absorption and bioavailability of the base scaffold with and without the amidine functionality.[16] Both compounds pass Lipinski's Rule of 5 and demonstrate acceptable values for molecular weight (MW < 350) and for calculated polar surface area (PSA < 125).[17,18] The amidine-containing analog demonstrated low oral absorption and bioavailability (<1%), while the corresponding non-amidino counterpart had improved oral absorption (~100%) and bioavailability (16%). This initial study in rats confirmed that the amidine hindered

Keywords: Factor VIIa; Anticoagulants; Thrombosis; Serine protease; Prodrug; Amidine; Amidoxime; Hydroxy amidine; Alkoxy amidine; Carbamate prodrugs; Pharmacokinetics; Indole synthesis.
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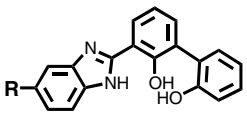
Table 1. Comparison of oral bioavailability for the amidino (**1**) and non-amidino (**2**) analogs in male rats[16]



Analog	R	VIIa K_i (μM)	PSA	MW	Abs (%)[a]	F (%)[b]
1[c]		0.41	119	344	<1	<1
2	H	37.0	69	302	~100	16

[a] Abs = oral absorption, based on portal-vein drug concentrations.
[b] F = oral bioavailability, based on jugular-vein drug concentrations.
[c] 2″-hydroxy-5″-fluoro analog, exact analog represented also had low % F.

oral absorption in this series. Following previous literature examples, we chose to utilize a prodrug strategy wherein the amidine was converted to hydroxy and alkoxy amidines. Typically, these prodrugs are converted back to parent upon absorption via a reductive cleavage of the amidine N–O bond within the liver.[19] The corresponding prodrugs of our series are less basic ($pK_a \sim 5$–7) and more lipophilic than the parent amidine and were envisioned to provide an increase in absorption and ultimately oral bioavailability of the corresponding parent amidine.

Hydroxy amidine **4** and a series of alkoxy amidines, including ethoxy, allyloxy, isopropoxy, and *tert*-butoxy (**5**–**8**), were synthesized and evaluated in vitro and in vivo (Table 2). The hydroxy amidine **4** showed good physical properties (PSA < 120, MW < 450), solubility, plasma stability, and rapid cleavage to parent in liver microsomes. The related alkoxy-amidine prodrugs (**5**–**8**) maintained good physical properties; however, solubility and cleavage in liver microsomes were reduced. These prodrugs were administered IV and PO to male Sprague–Dawley rats ($n = 3$ rats/compound per route) and the amount of parent and prodrug in plasma was quantified as a function of time.[16] As anticipated, all prodrugs showed moderate to high oral absorption and bioavailability; however, the percent of prodrug that was converted to parent following absorption (i.e., bioavailability of the parent) was negligible for all alkoxy prodrugs.

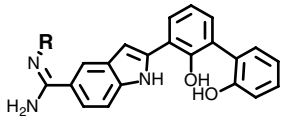
It has been suggested that pig enzymes are better models for the human enzymatic reductive processes which are required for prodrug conversion.[19] Hydroxy amidine **4** was evaluated in vitro in pig microsomes and in vivo in pigs, and the results paralleled those observed in rats.[20] In conclusion, both pigs and rats demonstrated low oral bioavailability due to negligible conversion of the hydroxy amidine prodrug to parent amidine after absorption.

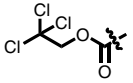
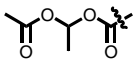
The low reductive cleavage that was obtained with our hydroxy- and alkoxy-amidine prodrugs led us to focus on prodrugs that could be converted to parent by an alternate mechanism, such as enzymatic hydrolysis or oxidation. To this end, we chose to evaluate amidino carbamates as prodrugs. The masking of amidines as carbamates has been used successfully in various aryl amidine cases to improve oral bioavailability.[21] To assess this prodrug strategy, a range of carbamate prodrugs (**9**–**12**) of base amidine **3** were evaluated both in vivo and in vitro (Table 2). The carbamates (**9**–**12**) exhibited acceptable PSA and MW values; however, the aqueous solubilities of these prodrugs were lower than those of the parent amidine or the corresponding hydroxy- and alkoxy-amidine prodrugs. The carbamates were stable in plasma (with the exception of **12** in rat) and in simulated gastric fluid,[22] minimizing the possibility of cleavage prior to absorption in vivo. Although the conversion of the carbamate prodrugs to parent amidine (i.e., 3–20%) was superior to the conversion of the hydroxy- and alkoxy-amidine prodrugs to parent (i.e., <1%) following IV administration in rats,[16] the oral bioavailability of the parent amidine after oral administration of these prodrugs was between 1% and 2% in all cases. As before, the low oral bioavailability of the parent amidine was due to low cleavage of the carbamate prodrug. With the outcome of our prodrug evaluation on our amidine fVIIa scaffold **3**, we changed our strategy and replaced the aryl-amidine P1 of **3** with a non-amidino heterocycle. The result of these efforts will be disclosed in a future publication.

The indole scaffold (**3**) was completed according to Scheme 1. The 2,2′-bisphenol **13** was mono-protected with MemCl and selectively *ortho*-formylated with paraformaldehyde and $MgCl_2$ using the conditions of Hofslokken et al. to yield aldehyde **14**.[23] Acid hydrolysis of the Mem group followed by protection of the bis-phenol as its bis-methyl ether with MeI yielded bis-anisole **15**. Conversion of the aryl aldehyde to the corresponding alkyne was accomplished using the diazophosphonate Ohira reagent **16**. The indole ring was constructed via a Sonagashira coupling between iodobenzonitrile **18** and alkyne **17** to produce a transient biaryl alkyne, which spontaneously cyclized upon the acidic mesylate nitrogen to produce the corresponding *N*-mesyl indole **19**. The indole nitrogen sulfonamide was removed via treatment with NaOH/MeOH. Pinner conversion of the nitrile intermediate to the ethyl imidate followed by treatment with ammonium carbonate provided the desired amidine. The compound was globally deprotected with aq HBr to cleave the methyl ethers to provide amidine **3**. Compound **3** was purified by preparative reverse-phase HPLC, and isolated as its corresponding HCl salt after lyophilization.

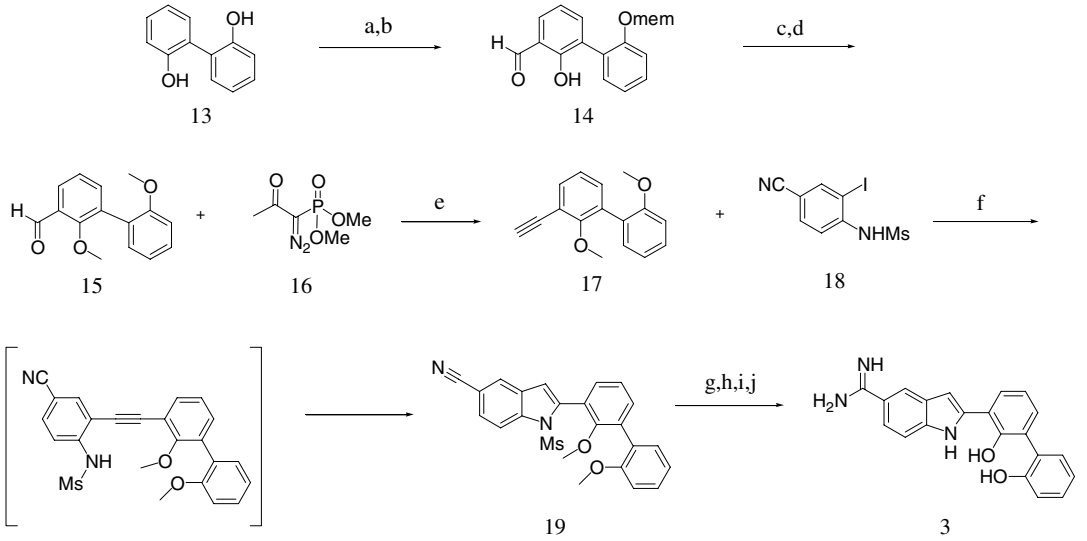
The synthesis of the hydroxy and alkoxy amidine prodrugs began with deprotection of bis methyl ether **20** with BBr_3 followed by Pinner conversion of the nitrile to the corresponding ethyl imidate (Scheme 2). Displacement of the imidate with either hydroxylamine to provide the hydroxyamidine or alkylhydroxyamines to produce alkoxyamidines completed the first series of prodrugs **21**. Conversely, the simple hydroxyamidine can be formed directly from the nitrile upon treatment with hydroxylamine. The carbamate prodrugs **23** were completed by reaction of *p*-nitrophenyl carbonate **22**

Table 2. Physical properties, stability, and pharmacokinetic parameters of parent amidine and hydroxy-, alkoxy-, and carbamate-amidine prodrugs

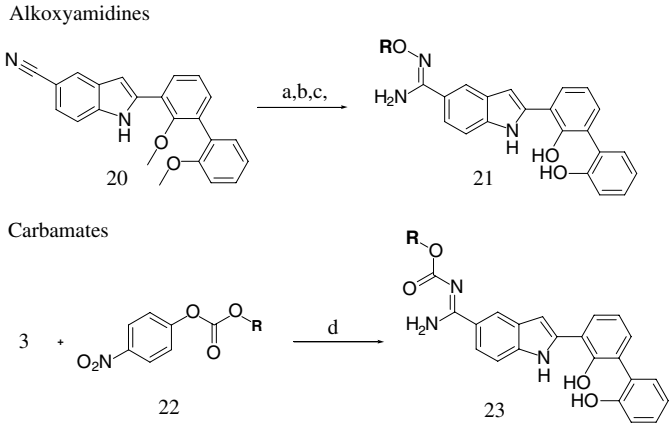


Analog	R	Calculated physical properties		Solubility[24] (μM)	Plasma stability[25] (% remaining at 2 h)		Liver microsome stability[26] (% remaining at 1 h)		PK parameters in rats		
									Prodrug		Parent
		PSA	MW	pH 7.4	Rat	Human	Rat	Human	Abs[a] (%)	F[b] (%)	F[b] (%)
3[c]	H	106	343	83	100	100	100	61	—	—	<1
4	OH	115	359	380	94	97	9	41	39–87	15–39	1
5		104	387	43	100	100	70	50	53–100	67–81	<1
6		104	399	24	94	100	42	55	100	100	2
7		104	401	25	100	100	69	63	54	10–36	<1
8		104	416	<10	99	100	78	100	60–100	38–62	<1
9		118	415	<10	95	100	53	37	ND[d]	ND[d]	2
10		118	429	<10	100	100	49	ND[d]	53–81	23	<1
11		118	519	<10	89	100	9	87	21–31	14	2
12		145	473	<10	37	96	12	9	ND[d]	ND[d]	2

[a] Abs = oral absorption, based on portal-vein drug concentrations.
[b] F = oral bioavailability, based on jugular-vein drug concentrations.
[c] 2″-hydroxy-5″-fluoro analog, exact analog represented also had low % F.
[d] ND, not determined.



Scheme 1. Synthesis of indole base scaffold **3**. Reagents and conditions: (a) Mem-Cl, K_2CO_3; (b) $MgCl_2$, TEA, Paraformaldehyde; (c) HCl/dioxane 10–30% over 3 steps; (d) MeI, NaH, 80%; (e) K_2CO_3, MeOH, 75%; (f) $PdCl_2(PPh_3)_2$, TEA, CuI; (g) NaOH, MeOH, reflux, 55% over 2 steps; (h) $HCl_{(g)}$, EtOH; (i) $(NH_4)_2CO_3$; (j) HBr, 40% over 3 steps.



Scheme 2. Synthesis of hydroxy, alkoxy amidines and carbamates. Reagents: (a) BBr_3; (b) $HCl_{(g)}$, EtOH; (c) **R**-ONH_2, 30–50% over 3 steps; (d) TEA, DMF, 20–40%.

with amidine **3**.[27] *p*-Nitrophenyl carbonates were made from the corresponding commercially available chloroformates.

The practice of employing amidine prodrugs as a means to improve oral bioavailability of amidine compounds has been a successful strategy in a number of relevant cases. Hydroxy and alkoxy amidines were made in our factor VIIa inhibitor scaffold to test our hypothesis for increased oral bioavailability. The hydroxy amidine showed low microsomal stability in vitro, suggesting good prodrug conversion was possible; however, negligible cleavage to the parent amidine was observed in vivo. Alternatively, the alkoxy amidines remained relatively stable both in vitro and in vivo, leading to moderate absorption of the prodrug, but very low conversion and thus very low oral bioavailability of the parent amidine.

Additionally, the carbamate prodrugs also remained very stable in both plasma and gastric fluid with the exception of the *N*-acyloxyalkoxy amidine **12** in rat plasma. The in vitro instability of analog **12** did not correlate with increased oral bioavailability of the parent amidine in vivo.

Many factors can affect the lack of conversion of our amidine prodrugs. Successful applications of amidine prodrugs have typically involved alkyl- and mono aryl-amidine structures,[28] unrelated to our biaryl scaffold system. We hypothesize that our lack of prodrug conversion to the parent amidine upon absorption is the result of our biaryl scaffold not being recognized as a substrate for the enzymes necessary to effect the amidine prodrug conversion.[20]

Acknowledgment

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25. Frozen, pooled human and rat plasma in sodium heparin were purchased from Bioreclamation (Hicksville, NY). Plasma stability was determined by incubating compound (10 μM) at 37 °C for 2 h. The disappearance of the prodrug was monitored by LC/MS/MS. The percentage remaining at 2 h was calculated by dividing the peak area of the prodrug at the end of the incubation by the peak area of the prodrug at the start (time 0).
26. Cryopreserved, pooled, male human and rat liver microsomes were purchased from Xenotech, LLC (Lenexa, Kansas). Liver microsome stability was determined by incubating compound (2 μM) at 37 °C for 1 h. The disappearance of the prodrug was monitored by LC/MS/MS. The percentage remaining at 1 h was calculated by dividing the peak area of the prodrug at the end of the incubation by the peak area of the prodrug at the start (time 0).
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