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NOVEL BENZO-FUSED LACTAM SCAFFOLDS AS FACTOR Xa INHIBITORS

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Abstract: Rigid benzolactam P_3–P_2 dipeptide mimics were designed and prepared as potential inhibitors of blood coagulation factor Xa. Methoxy substitution of the tetrahydrobenzazepinone scaffold led to potent and selective inhibitors. The synthesis and biological activities of these derivatives are reported herein.

Factor Xa is a critical enzymatic mediator of the coagulation response to vascular injury and regulates normal hemostasis and abnormal thrombus development.[1] As a key member of the trypsin class of serine proteases involved in blood coagulation, it catalyzes the penultimate step in this biochemical cascade, the formation of the serine protease thrombin. Factor Xa-mediated thrombin formation occurs following the assembly of the prothrombinase complex, which is composed of factor Xa and the non-enzymatic cofactor factor Va assembled on an appropriate phospholipid surface such as activated platelets. Preclinical studies with selective inhibitors of prothrombinase activity in relevant models of thrombosis have provided the necessary experimental evidence to support the development of a new generation of antithrombotic agents based on inhibition of factor Xa.[2] A variety of small molecule synthetic scaffolds that selectively inhibit factor Xa as well as prothombinase activity are currently receiving considerable attention and are emerging as potential therapeutic agents for the prevention and treatment of thrombotic vascular disease.[3]

Our work on oral thrombin inhibitors led to the discovery of **1**, a potent mixed inhibitor of both thrombin and factor Xa (IC_{50}'s of 0.71 nM and 20.6 nM, respectively).[4] The design of peptidomimetic inhibitors that feature lactam and related heterocyclic motifs is currently an area of active investigation. Conformationally constrained dipeptide mimetics can contribute a favorable entropic component to their binding relative to their highly flexible peptide counterparts. The design of targets which incorporate tetrahydrobenzazepinones and dihydroquinolinones as rigid P_3-P_2 probes (Figure 1) was based on the topography[5]

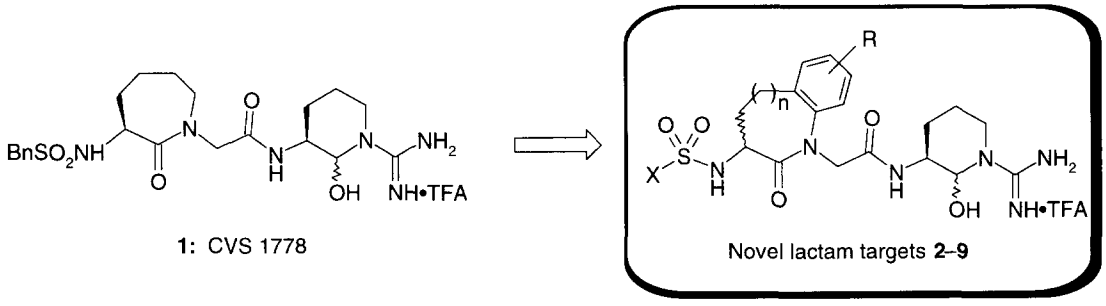

Figure 1. Strategy for the design of benzolactam based inhibitors, **2–9**.

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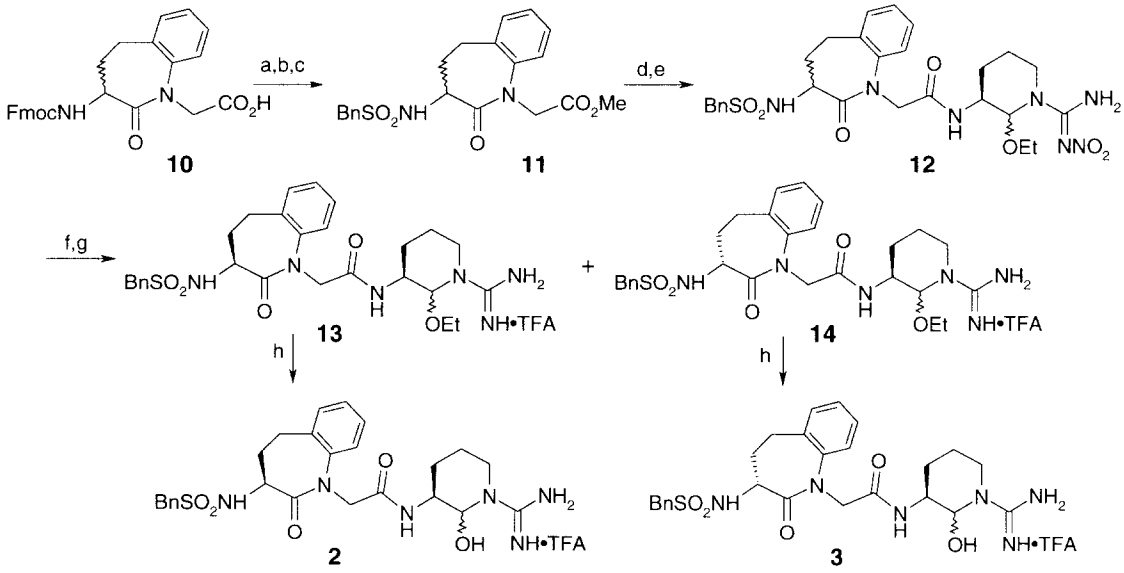
and putative flexibility[6] of the active site of factor Xa that may impart a capacity for the S_2 pocket to accommodate large hydrophobic moieties. These scaffolds have restricted dihedral angles and conformations, conferring improved structural features that help stabilize the requisite conformation of a potential inhibitor in the active site of the enzyme. The targets retain a P_1-argininal moiety, an electrophilic transition-state functionality that often imparts useful levels of oral bioavailability to inhibitors of trypsin-like serine proteases.[4] The synthesis and biological activity of this novel family of benzo-fused lactams is described in this letter.

Synthetic Route to Inhibitors 2 and 3[7]

The synthesis of the two isomeric targets, **2** and **3**, was accomplished in eight steps from the commercially available intermediate **10**[8] (Scheme 1). Esterification of **10** via the imidazolide, followed by Fmoc deprotection and reaction with α-toluenesulfonyl chloride produced **11** in good overall yield. Saponification of **11** yielded the corresponding acid, which was coupled to nitroargininal ethyl aminal,[9] to afford **12**. After hydrogenolysis and separation by preparative RP HPLC, the two P_3 diastereoisomers **13** and **14** were isolated. Each isomer, **13** and **14**, was converted to its corresponding tetrahydrobenzazepinone-argininal targets **2** and **3** by mild acid hydrolysis of the aminal. The stereochemistry of each isomer was unambiguously confirmed by synthesis of compound **3** from α-tetralone using resolution protocols similar to those described below for compounds **4–6**, only substituting D-pyroglutamic acid for L-pyroglutamic acid during the resolution of the (*R*)-3-amino-tetrahydrobenzazepinone intermediate (vide infra).[10]

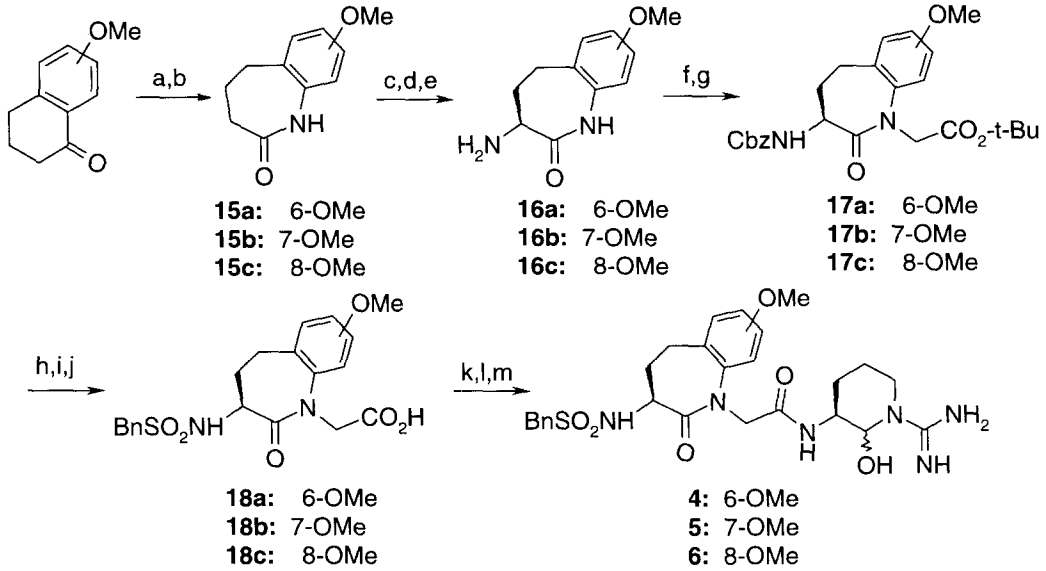


Scheme 1. *Reagents and conditions:* (a) CDI, CH_3CN, rt; MeOH, 75%; (b) Et_2NH, CH_2Cl_2, rt, 84%; (c) α-toluenesulfonyl chloride, Et_3N, CH_3CN, 68%; (d) LiOH, aq MeOH, 95%; (e) EDC, HOBt, nitroargininal ethyl aminal•HCl, NMM, CH_3CN, 52%; (f) H_2, 10% Pd/C, EtOH, H_2O, HOAc; (g) preparative RP HPLC, 21% for **13**, 42% for **14**; (h) 6.0 N HCl, CH_3CN; RP HPLC, 38% for **2**, 17% for **3**.

Synthetic Route to Inhibitors 4–6

The preparation of compounds **4–6** was accomplished in 13 steps via modification of resolution protocols[9] to produce (*S*)-amino-tetrahydrobenzazepinones **16a–c** (see Scheme 2). The oximes of methoxy-α-

tetralone underwent Beckman rearrangement to give a mixture of the two isomeric lactams from which compounds **15a–c** were isolated after flash chromatography. The lactams were iodinated using the methodology described by Armstrong et al.[11] Since the standard displacement of the iodo-lactam with sodium azide was problematic, simple displacement with ammonia was attempted. Conversion to the amine using saturated ammonia in aqueous ethanol was sluggish, but occurred without the production of any side products. Resolution of the racemic amines was performed to afford compounds **16a–c** using a modification of the methodology described by Fischer et al.[10,12] After protection of the amines **16a–c**, selective N-alkylation of the lactam ring nitrogen with *t*-butyl bromoacetate provided **17a–c**. Hydrogenolysis of the Cbz moiety, formation of the sulfonamide, and subsequent removal of the *t*-butyl ester afforded acids **18a–c**. A three-step protocol was utilized for the final assembly of tetrahydrobenzazepinone targets **4–6**: coupling of the tetrahydrobenzazepinone acid synthons **18a–c** to nitroargininal ethyl aminal, hydrogenolysis of the nitro group from the guanidine, and mild acidic hydrolysis to unmask the aldehyde.

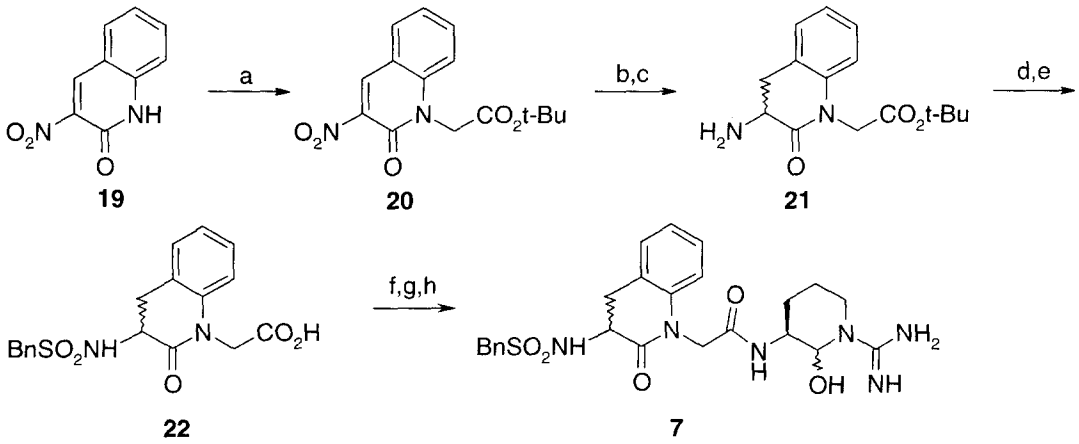


Scheme 2. *Reagents and conditions:* (a) NH_2OH, NaOAc, EtOH, rt–50 °C, 90-100%; (b) PPA, 115 °C, 5 min, 16-80%; (c) TMSCl, TMEDA, NaI, CH_3CN; –5 °C, 20 min, I_2, 86–94%; (d) NH_3, aq EtOH, 61–86%; (e) 95% aq *i*-PrOH, L-pyroglutamic acid, reflux; conc. NH_3, 58–100%; (f) Cbz-OSu, $NaHCO_3$, aq dioxane, 92–99%; (g) $LiN(TMS)_2$, *t*-butyl bromoacetate, THF, 0 °C–rt, 77–95%; (h) 15 psi H_2, 10% Pd/C, EtOH, 94–100%; (i) $BnSO_2Cl$, NMM, THF, 80–100%; (j) TFA, CH_2Cl_2, quantitative; (k) EDC, HOBt, nitroArg-al ethyl aminal•HCl, CH_3CN, NMM, 48–54%; (l) 40 psi H_2, 10% Pd/C, EtOH, HOAc, H_2O, quantitative; (m) 4 N HCl; preparative RP HPLC purification, 35–55%.

Synthetic Route to Inhibitor 7

The preparation of the dihydroquinolone target **7** was accomplished in eight steps from the known 3-nitroquinolinone **19**.[13] Alkylation of **19** with *t*-butyl bromoacetate delivered **20**. Conjugate reduction of **20** with sodium borohydride reduction, followed by hydrogenation of the nitro group, produced compound **21**. Formation of the benzylsulfonamide and cleavage of the *t*-butyl ester afforded the tripeptide surrogate **22**. The final conversion to target **7** was accomplished in the same manner as for the compounds **4–6**: coupling of the dihydroquinolinone acetic acid **22** to nitroargininal ethyl aminal[9] selective hydrogenolysis of the nitro group

from the guanidine, and mild acidic hydrolysis to unmask the aldehyde. Unlike the diastereomeric tetrahydrobenzazepinone pairs **2** and **3**, the diastereomeric dihydroquinolinone system **7** was inseparable during all stages of the synthesis.



Scheme 3. *Reagents and conditions:* (a) K_2CO_3, *t*-butyl bromoacetate, acetone, reflux, 5 h, 74%; (b) $NaBH_4$, EtOH, THF, 60%; (c) H_2, 10% Pd/C, MeOH, 52%; (d) $BnSO_2Cl$, NMM; (e) TFA, CH_2Cl_2, 96%; (f) EDC, HOBt, nitroArgal ethyl aminal•HCl, CH_3CN; NMM, 71%; (g) 40 psi H_2, 10% Pd/C, EtOH, HOAc, H_2O; (h) 4 N HCl; preparative RP HPLC purification, 39% for 2 steps.

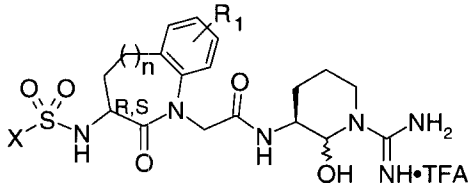
Results and Discussion

Eight benzo-fused lactam argininals were prepared and evaluated for their ability to inhibit four serine proteases (Table 1).[14] Potent and selective inhibitors of factor Xa were discovered that also displayed favorable selectivity against the fibrinolytic protease plasmin. It was demonstrated that the (*S*)-α-lactam stereochemistry was required for maximizal inhibitory potency against factor Xa: stereoisomer **2** was approximately seven-fold more potent than **3** against free factor Xa. Surprisingly, the (*R*)-isomer **3** was a kinetically fast inhibitor, while the (*S*) isomer **2** was a slow-binding inhibitor of factor Xa,[15] typical of this argininal class of transition-state inhibitors. The potency of **2** indicates that the benzo moiety is able to fit into the S_2 site of factor Xa suggesting there may be flexibility in this subsite; moreover, the selectivity of compound **2** against thrombin relative to compound **1** may be due to unfavorable steric interactions of the benzo moiety with the 60 loop of thrombin. 7-Methoxytetrahydrobenzazepinone-argininal **5** exhibited potency against factor Xa (7.2 nM) and enhanced selectivity against thrombin (>350-fold) and trypsin (15-fold) relative to the other compounds listed. The increased selectivity of compounds **5** and **6** against thrombin may be due to additional adverse steric interactions of the methoxy substituents with the 60 loop of thrombin.[16] Alternate P_4 substituents replacing the benzyl sulfonamide of compound **5**, as exemplified in compounds **8** and **9**, resulted in more potent inhibitors of factor Xa , but also showed less favorable thrombin and/or trypsin selectivities than the parent. Remarkably, decreasing the lactam ring size to a six-membered ring resulted in a dramatic change in both activity and protease selectivity; although a strong inhibitor of thrombin, the dihydroquinolinone **7** showed little potency against factor Xa.

The assembly of the prothrombinase complex results in specific structural and kinetic changes in factor Xa, resulting in a 100,000-fold improvement in the catalytic efficiency of prothrombin activation by factor Xa.[17] The potency of the described inhibitors was therefore measured against the natural substrate prothrombin by the

prothrombinase complex (Table 2). In all analogs examined, the potency of the inhibitor candidates against the prothrombinase complex as measured using the IC_{50} were two- to fivefold higher than the corresponding results using the amidolytic substrate with the uncomplexed factor Xa. Interestingly, compound **9**, which is the analog of compound **5** that contains a saturated P_4 substituent, resulted in a threefold enhancement in potency against both free factor Xa and prothrombinase.

Table 1. In Vitro IC_{50} Values (nM) of Peptide Mimics Against Human Factor Xa, Thrombin, Plasmin, and Trypsin[a]



compound	n	(configuration)	X	R_1	factor Xa	thrombin	plasmin	trypsin
2	1	(*S*)	Bn	H	7.3	178	>2500	26
3	1	(*R*)	Bn	H	49[b]	192	>2500	135
4	1	(*S*)	Bn	6-OMe	8.6	235	>2500	77
5	1	(*S*)	Bn	7-OMe	7.2	>2500	>2500	110
6	1	(*S*)	Bn	8-OMe	28.9	>2500	>2500	40
7	0	(*R,S*)	Bn	H	>2500	17.9	>2500	289
8	1	(*S*)	Naphthyl	7-OMe	3.6	285	>2500	27
9	1	(*S*)	CyclohexylCH_2	7-OMe	1.9	829	>2500	1.5
reference standard:								
1		(*S*)			20.6	0.71	>2500	75

[a]Concentration of compounds **1–9** necessary to inhibit human enzymes (factor Xa, factor IIa, plasmin, and trypsin) by the cleavage of the chromogenic substrates described in ref 4a by 50%. [b]Estimated K_i for classical fast inhibition.[18]

Table 2. In Vitro IC_{50} Values (nM) of Inhibitors Against Uncomplexed Factor Xa[a] and Prothrombinase[b]

compound	factor Xa	prothrombinase	ratio FXa/PTase
2	7.3	17.1	2.3
4	8.6	26.7	3.1
5	7.2	34.9	4.8
6	28.9	64.3	2.2
8	3.6	19.1	5.3
9	1.9	9.9	5.2

[a]Concentration of compounds **2**, **4–6**, **8–9** necessary to inhibit the cleavage of the chromogenic substrate by human enzyme factor Xa described in ref 4a by 50%. [b]Concentration of compounds **2**, **4–6**, **8–9** necessary to inhibit the activation of human prothrombin in a preformed complex of factor Xa, factor Va and phospholipid vesicles as described in ref 19 by 50%.

Conclusion

A novel series of benzolactam dipeptide surrogates was appended onto a P_1-argininal moiety resulting in the discovery of a series of potent and selective factor Xa inhibitors. Of these new compounds, compound **5** expressed favorable in vitro potencies against uncomplexed factor Xa and against the prothrombinase complex, with desirable selectivity towards other trypsin-like serine proteases. The versatile sulfonamide/heterocycle motif imparts intriguing activity and selectivity profiles toward various target enzymes. The further investigation of their applications will be reported in due course.

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