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Design and synthesis of bicyclic pyrazinone and pyrimidinone amides as potent TF–FVIIa inhibitors

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ABSTRACT

Bicyclic pyrazinone and pyrimidinone amides were designed and synthesized as potent TF–FVIIa inhibitors. SAR demonstrated that the S2 and S3 pockets of FVIIa prefer to bind small, lipophilic groups. An X-ray crystal structure of optimized compound **9b** bound in the active site of FVIIa showed that the bicyclic scaffold provides 5 hydrogen bonding interactions in addition to projecting groups for interactions within the S1, S2 and S3 pockets. Compound **9b** showed excellent FVIIa potency, good selectivity against FIXa, Xa, XIa and chymotrypsin, and good clotting activity.

Vascular injury or atherosclerotic plaque rupture results in exposure of tissue factor (TF) to circulating factor VIIa (FVIIa) in plasma. Binding of TF to FVIIa leads to a large enhancement of the catalytic activity of FVIIa. The TF–FVIIa complex then initiates the extrinsic coagulation pathway by activating factor IX to IXa and factor X to Xa, which in turn activates prothrombin to thrombin. Thrombin cleaves fibrinogen to fibrin, which forms blood clots with activated platelets.[1,2] Inappropriate clot formation in blood vessels causes cardiovascular disease such as stroke and myocardial infarction. Recent preclinical studies have shown that selective inhibition of TF–FVIIa is effective in anticoagulation with a low risk of bleeding.[3–6] Herein, we report our discovery of bicyclic pyrazinone and pyrimidinone amides as potent TF–FVIIa inhibitors.

Based on knowledge that serine proteases typically bind their peptide substrate in an extended conformation and our previous work on 3-aminobicyclic pyrazinone and pyrimidinone as β-sheet mimetics,[7–9] we reasoned that by attaching an aminomethyl benzamidine to interact with Asp189 in the S1 pocket to the bicyclic pyrazinone or pyrimidinone scaffold, a series of potent TF–FVIIa inhibitors may be obtained (Fig. 1). Further supporting this proposal is the report by South et al. that mono-cyclic pyrazinone amides are potent TF–FVIIa inhibitors.[10–12] Bicyclic pyrazinone amide **1** was thus prepared and found to be a potent TF–FVIIa inhibitor with K_i of 30 nM (Fig. 1). Although compound **1** showed the same level activity against thrombin, it was highly selective against FXa. We considered it to be a good lead that warranted further investigation.[13]

The general synthesis of 3-amino bicyclic pyrazinone amides is shown in Scheme 1. Boc-protected pyroglutamate **2** was treated with lithium bis(trimethysilyl)amide and alkylated with an electrophile to give a 4-substituted pyroglutamate. This alkylation procedure is stereoselective and can be carried out twice to give the 4,4-disubstituted derivatives **3**.[14] The second electrophile reacts with the enolate preferentially *trans* to the 2-carboxylate, allowing stereocontrol of R^1 and R^2 simply by changing the order of addition of the electrophiles. Pyroglutamate **3** was reduced with lithium triethylborohydride to the aminal, which was converted to acetoxy derivative **4.** Intermediate **4** reacted with trimethylsilyl cyanide catalyzed by $BF_3{\cdot}OEt_2$ in methylene chloride to give 5-cyano proline derivative **5**. The Boc protecting group was removed to generate aminonitrile salt **6,** which underwent cyclocondensation with oxalyl chloride to give dichloropyrazinone **7**.[7] Heating a solution of dichloropyrazinone **7** in ethyl acetate in the presence of excess amine R^3NH_2 resulted in a selective nucleophilic displacement of the C-3 chlorine to give intermediate **8**. Saponification of compound **8** resulted in the acid, which was coupled with benzyl (4-(aminomethyl)phenyl)(imino)methylcarbamate. After removal of the Cbz group, compound **9** was obtained for biological testing.

Scheme 2 describes the preparation of bicyclic pyrimidinones. In this series, the *N*-terminal substituent was maintained as a propyl group since it was shown to be a preferred substituent in the pyrazinone series. Pyrimidinone **10**[8] was N-allylated and the carboxylic acid was masked as a phenyl amide **11**. Compound **11**

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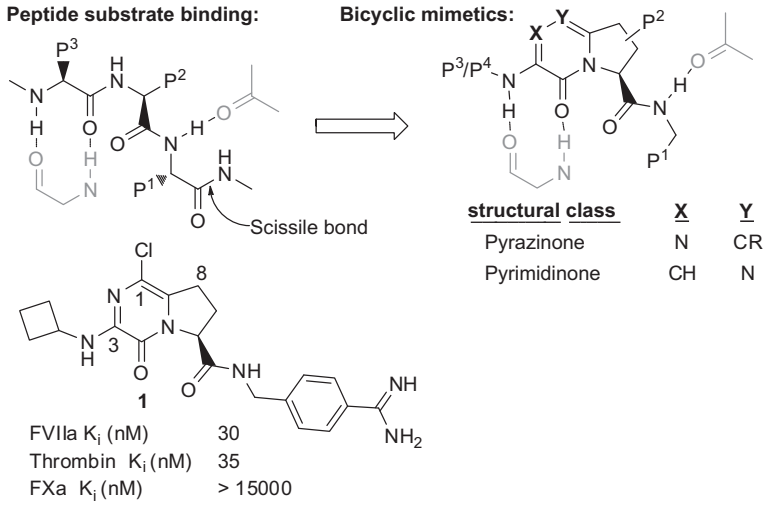
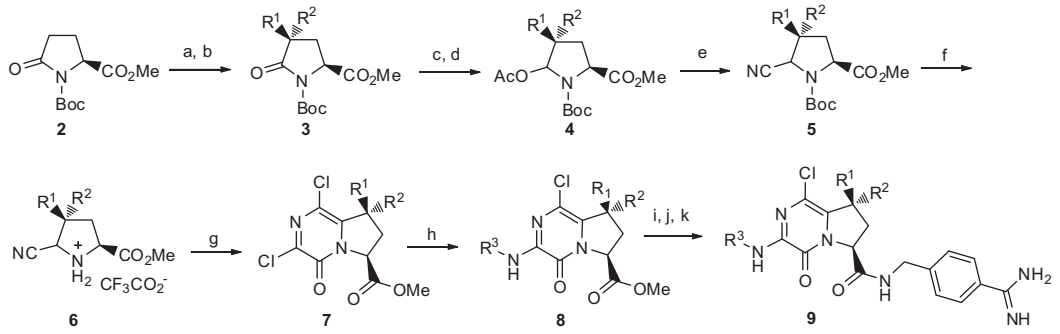
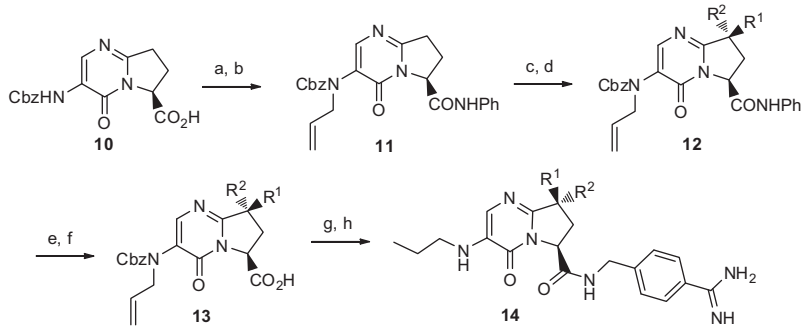


Figure 1. Design of bicyclic pyrazinone and pyrimidinone FVIIa inhibitors and initial lead **1**.



Scheme 1. Reagents and conditions: (a) LHMDS, THF, −78 °C, R^1-X; (b) LHMDS, THF, −78 °C, R^2-X, 30–90%, 2 steps; (c) $LiBHEt_3$, THF, −78 °C; (d) Ac_2O, −78 °C to rt, 50–90%, 2 steps; (e) TMSCN, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 °C, 50–70%; (f) TFA, CH_2Cl_2, >80%; (g) $(COCl)_2$, toluene, 85 °C, 25–70%; (h) R^3NH_2, EtOAc, 80 °C, >65%; (i) LiOH, THF, H_2O; (j) IBCF, NMM, THF, −20 °C, benzyl ((4 (aminomethyl)phenyl)(imino)methyl)carbamate; (k) H_2, Pd/C, MeOH, 50–90%.



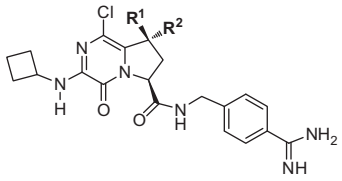
Scheme 2. Reagents and conditions: (a) NaH, AllylBr; (b) $PhNH_2$, EDC, HOAt, 58%, 2 steps; (c) LHMDS, R^1-X, (d) LHMDS, R^2-X, 40–95%, 2 steps, (e) Boc_2O, DMAP; (f) LiOOH, 80–91%, 2 steps; (g) EDC, HOAt, benzyl ((4-(aminomethyl)phenyl)(imino)methyl)carbamate; (h) H_2, Pd/C, MeOH, 50–90%, 2 steps.

was doubly alkylated stereoselectively to give intermediate **12**. Compound **12** was activated to the imide with Boc_2O and then hydrolyzed to acid **13**. Coupling of compound **13** with benzyl (4-(aminomethyl)phenyl)(imino)methylcarbamate and hydrogenolysis to remove the Cbz group and reduce the allyl to propyl gave final compound **14**.

We believed that the R^2 group at 8-position of the bicyclic pyrazinone should occupy the S2 pocket in the FVIIa active site based on modeling studies and analogy to binding of this chemotype to HCV NS3 serine protease, another chymotrypsin-like serine protease.[7] We expected the potency of compound **1** should be further improved with an optimal R^2 group engaging interactions in the S2 pocket. The SAR of compounds with varied R^1 and R^2 groups is summarized in Table 1.

It is clear that a small hydrophobic group is preferred at R^2. An allyl group (**9c**) is optimal, followed by ethyl (**9b**). Both compound **9b** and **9c** showed improved binding for FVIIa compared to compound **1**. Selectivity of compound **9b** against thrombin is also improved compared to compound **1**, and it remains more than 450-fold selective against FXa. Polar R^2 groups are not well

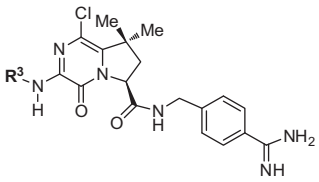
Table 1
Bicyclic pyrazinone-R_1 and R_2 SAR

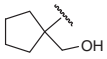
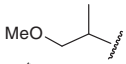
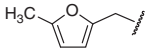


Cmpd	R^1	R^2	FVIIa K_i (nM)	Thrombin K_i (nM)	FXa K_i (nM)
1	H	H	30	35	>15,000
9a	Me	Me	23	200	>15,000
9b	**Et**	**Et**	**7.3**	**210**	**3300**
9c	**Me**	**Allyl**	**5.8**	**220**	**650**
9d	Me	*n*-Pr	23	36	1000
9e	*n*-Pr	Me	21	110	2200
9f	*n*-Pr	*n*-Pr	28	170	550
9g	$-CH_2CH_2CH_2CH_2-$		99	78	>15,000
9h	Me	CH_2OH	72	>15,000	>15,000
9i	Me	CONHMe	2100	>15,000	1100
9j	Me	CONHBn	560	>15,000	3700

K_i's for the indicated enzymes were determined by chromogenic substrate assays at 25 °C ($n = 2$).[15]

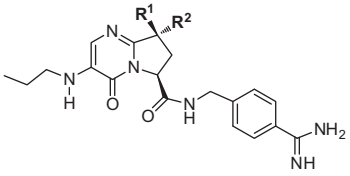
Table 2
Bicyclic pyrazinone-R^3 SAR



Compd	R^3	FVIIa K_i (nM)	Thrombin K_i (nM)	FXa K_i (nM)
9k	Me	24	230	>15,000
9l	Et	19	260	>15,000
9m	*i*-Pr	17	130	>15,000
9a	*c*-Bu	23	200	>15,000
9n	*c*-Pr	37	360	>15,000
9o	*i*-Bu	43	220	>15,000
9p	*c*-Pentyl	60	160	>15,000
9q	*t*-Bu	68	220	2800
9r		70	380	>15,000
9s		76	220	>15,000
9t		84	ND	ND
9u		130	ND	ND
9v	$Bu^tO_2CCH_2-$	132	32	290
9w		190	ND	ND

K_i's for the indicated enzymes were determined by chromogenic substrate assays at 25 °C ($n = 2$).[15]

Table 3
Bicyclic pyrimidinone-R^1 and R^2 SAR



Compd	R^1	R^2	FVIIa K_i (nM)	Thrombin K_i (nM)	FXa K_i (nM)
14a	H	H	186	150	>15,000
14b	Me	Me	290	1800	>15,000
14c	**Et**	**Et**	**75**	**5200**	**12,000**
14d	Me	Et	150	2100	>15,000
14e	*n*-Pr	*n*-Pr	620	1200	3600

K_i's for the indicated enzymes were determined by chromogenic substrate assays at 25 °C ($n = 2$).[15]

tolerated, as seen with hydroxy methyl (**9h**) and amides (**9i** and **9j**). A spiro compound **9g** was synthesized to further constrain the diethyl side chains, however it was found to be less active than compound **9b**.

SAR of substituents R^3 on the amino terminus has been investigated using dimethyl groups as the P2 side chain for synthetic simplicity. The results are summarized in Table 2. Similar to the observation in the monocyclic pyrazinones reported by South et al.,[11,12] small alkyls, such as Me (**9k**), Et (**9l**), *i*-Pr (**9m**), *c*-Bu (**9a**) and *c*-Pr (**9n**) were among the best groups. FVIIa binding affinity decreased as the size of R^3 group increased (**9o**–**w**) beyond *c*-Bu and *i*-Pr. The R^3 group did not seem to affect the selectivity against thrombin and FXa.

A number of bicyclic pyrimidinone amides have also been prepared, varying the R^1 and R^2 groups. In general, the SAR follows the trend observed in the pyrazinone series, with diethyl as the preferred P2 group (Table 3). Bicyclic pyrimidinone amides are less potent than the pyrazinone amides, likely due to the absence of the chlorine substituent on the ring. The des-chloro analog of compound **1** in the pyrazinone amide series is 2-fold less potent than the parent compound **1** (data not shown), suggesting the beneficial effect of the chlorine on binding.

Compound **9b** has been further characterized because of its excellent potency. It showed very good selectivity (>160-fold) against FIXa, Xa, XIa and chymotrypsin and moderate selectivity (30-fold) against thrombin. However, selectivity against trypsin and plasma kallikrein is limited (<3-fold). Free fraction of compound **9b** in human plasma is 6.8%. It is efficacious in a human plasma clotting assay with FVII deficient PT of 5.6 μM.[15] Attempts to replace the highly basic benzamidine P1 in compound **9b** (Fig. 2) with a less basic amine such as benzyl amine (**15**), 1-aminoisoquinonline (**16**) and

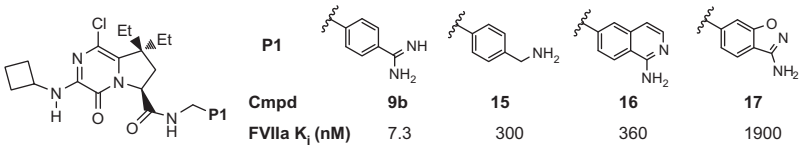


Figure 2. Replacement of bezamidine in compound **9b** with weakly basic amines.

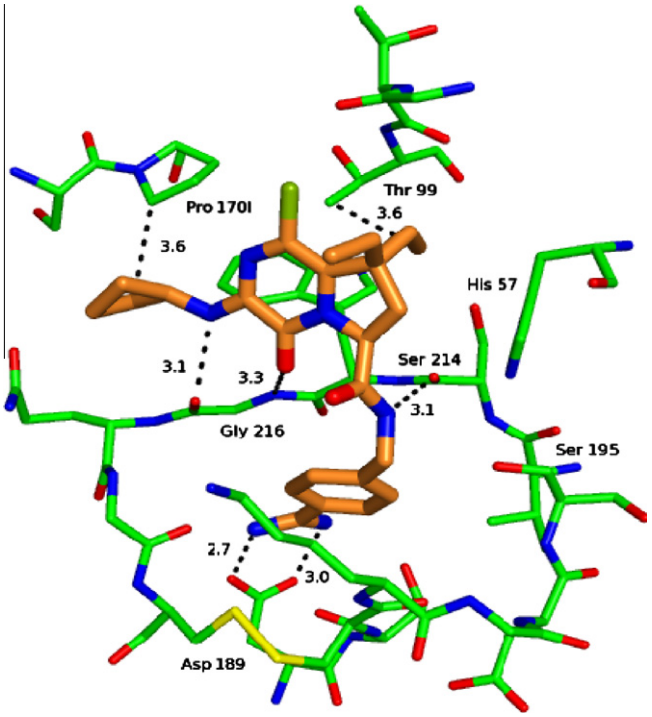


Figure 3. X-ray crystal structure of compound **9b** bound in the active site of FVIIa.

3-aminobenzoisoxazole (**17**) all resulted in significant loss (40–260-fold) of potency. One of the major challenges in the development of drugs inhibiting TF–VIIa is attaining oral bioavalability. Only limited reports of non-benzamidine TF–FVIIa inhibitors have recently appeared in literature.[16–18]

An X-ray crystal structure of compound **9b** bound to FVIIa was solved (Fig. 3) to better understand how these bicyclic pyrazinone amides bind in the active site of the enzyme.[20] As we anticipated, besides the key salt bridge interactions of the benzamidine with Asp189 in the bottom of the S1 pocket, the pyrazinone scaffold provided three additional key hydrogen bond interactions with the enzyme backbone: a pair from the pyrazinone carbonyl and terminal NH to interact with Gly216, and the amide NH to interact with Ser214 hydroxyl. The R^2-ethyl group engages in a hydrophobic interaction with the side chain methyl of Thr99 in the S2 pocket while R^1 group is solvent exposed. This R^2-ethyl group also displaces a structural water molecule from the S2 pocket that is often seen in other TF–FVIIa/inhibitor crystal structures.[19] The cyclobutyl group attached to the terminal nitrogen makes hydrophobic interactions with Pro170I.

In summary, we have designed and synthesized bicyclic pyrazinone and pyrimidinone amides as potent TF–FVIIa inhibitors. SAR shows that the S2 and S3 pocket of the enzyme prefer to bind small, lipophilic groups. The bicyclic scaffolds provide three hydrogen bonding interactions in addition to projecting groups for interactions within the S1, S2 and S3 pockets. Compound **9b** showed excellent FVIIa potency, good selectivity against FIXa, Xa, XIa and chymotrypsin and good clotting activity.

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