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Ketene aminal-based lactam derivatives as a novel class of orally active FXa inhibitors

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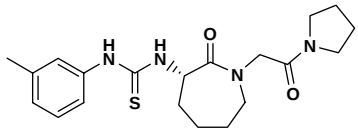
In memory of Dr. Steven M. Seiler.

Abstract—*N*,*N*′-Disubstituted ketene aminals are good bioisosteres of thiourea functional groups. We report the design and synthesis of a novel class of ketene aminal-based lactam derivatives as potent and orally active FXa inhibitors.

Inhibition of the trypsin-like serine protease factor Xa (FXa) has emerged as a key point of intervention in the blood coagulation cascade for the development of antithrombotic agents.[1] Within the cascade, factor Xa functions at the point where the intrinsic and extrinsic coagulation pathways converge,[2] and FXa is the key enzyme responsible for thrombin activation.

Factor Xa contains a deep S1 and a box-like S4 recognition site at the enzyme's active site. Potent FXa inhibitors generally require both an S1 and an S4 binding element which are connected through L-shaped or other 'bent' scaffolds.[1] Many factor Xa inhibitors in the literature contain basic pharmacophores such as guanidine or amidine as S1 or S4 binding substrates; their pharmacokinetic profiles are thus limited due to low oral bioavailability and short plasma half life. We have been interested in the development of novel, orally bioavailable FXa inhibitors.[3] As a result of our early program effort,[4] it was found that compound **1** is an inhibitor of human FXa (IC_{50} = 110 nM). Although it contains a thiourea, its novel structure is intriguing due to the absence of an active site directing guanidine or amidine moiety in S1, and its nonpeptidic nature. Also, compound **1** was found to be relatively selective for FXa when tested against a panel of trypsin-like serine proteases.[4] Based on this lead, we embarked on synthetic efforts to explore alternate bioisosteres to the thiourea motif that could adopt a L-shaped conformation and would be more chemically stable and amenable to a final drug candidate. Herein, we describe a series of ketene aminal-based lactam derivatives as a novel class of orally bioavailable FXa inhibitors.[5]



1 IC_{50} = 110 nM

Keywords: Serine protease; Factor Xa inhibitors; Antithrombotic agents; 2-Methylbenzofuran; Thiourea; Bioisosteres; *N*,*N*′-Disubstituted ketene aminal; 2,2-Dicyanoketene aminal; 2-Nitroketene aminal; Orally active.
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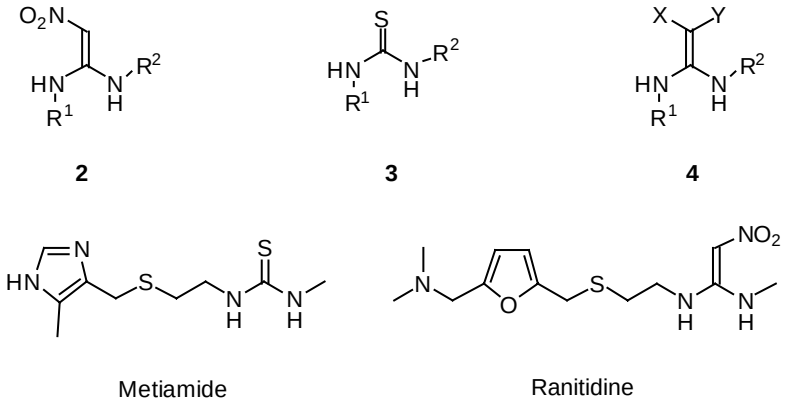
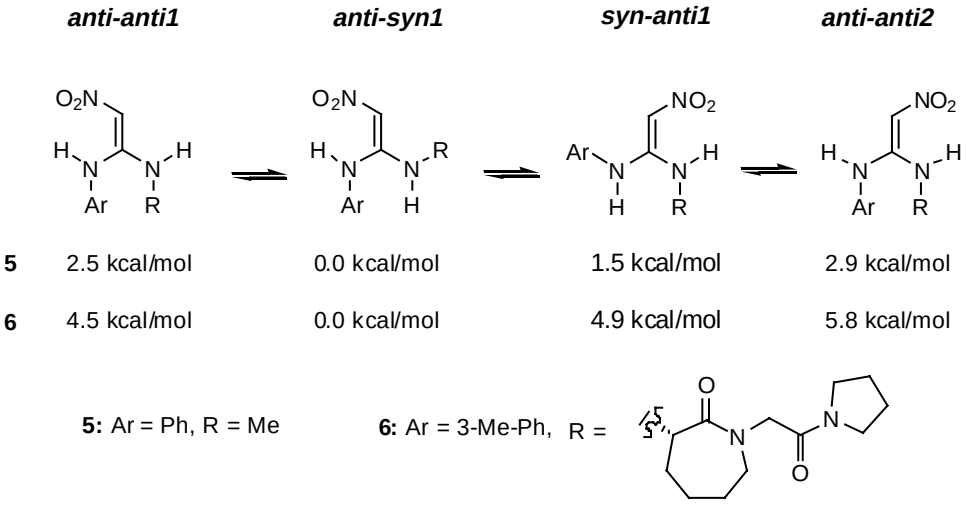
The *N*,*N*′-disubstituted 2-nitroketene aminal **2** is known to be an excellent bioisostere of *N*,*N*′-disubstituted thiourea **3** in histamine H_2-receptor antagonist studies.[6] For example, the nitroketene aminal in Ranitidine[7] is a bioisostere of the thiourea functional group in Metiamide[8] (Scheme 1). Similar to its thiourea analog **1**,[4] (*S*)-3-(1-(*m*-toluidino)-2-nitrovinylamino)-1-(2-oxo-2-(pyrrolidin-1-yl)ethyl))azepan-2-one (compound **6**), is predicted to prefer the *anti–syn1* conformation by gas-phase semi-empirical calculations.[9] Scheme 2 shows the four lowest energy conformers for both **6** and *N*-phenyl-*N*′-methyl 2-nitroketene aminal **5**. The other possible conformers are disfavored due to the internal steric interactions between nitrogen substituents and the nitro group. It is clear that both compounds prefer *anti–syn1* conformation with the aromatic group (Ar) *anti* to the nitroketene group and the alkyl group (R) *syn* to the ketene. This conformation affords the best combination of relief from steric crowding between the nitro and the Ar or R groups, and the stabilization by intramolecular hydrogen bonding between the nitro group and an aminal NH. The *anti–anti* conformations are disfavored because of steric interactions between the R and Ar groups. The *syn–anti1* conformation is less favored than *anti–syn1* probably due to the steric interaction of the R group and the hydrogen atom from Ar-N–H. This effect becomes more pronounced for **6** with a bulkier R group relative to **5**. In addition, the hydrogen bonding between the nitro group and the aryl N–H enhances the planarity of the aryl N–H by making it part of a 6-membered hydrogen bond ring, whereas when the nitro is hydrogen bonded to the alkyl N–H, the Ar-N–H nitrogen becomes more pyramidal. Thus, the Ar-N–H groups in *anti–syn1* conformers have better conjugation with the ketene group than their *syn–anti1* conformers.

Electronically, both thiourea[10] and ketene aminal[11] have large dipole moments that have been attributed to considerable resonance contributions from dipole canonical structures, which allows the ketene aminal to exist in a rapid rotameric equilibrium around the ketene double bond in polar solvents.[12] Thus, the ketene double bond can adopt two conformations in binding to enzymes. Electron-withdrawing substituents on the ketene group (X and Y in **4**) also behave similarly to the thione carbonyl (S=C) by reducing the electron density of the conjugated amino groups. As a result, the ketene aminal serves as a neutral bio-isostere to thiourea.[6b,13]



Scheme 1. The thioureas and ketene aminals.

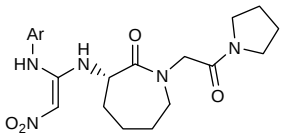


Scheme 2. Relative energies of conformers of compounds **5** and **6**.

Early efforts in our FXa program focused on analogs of **1** where the thiourea moiety is replaced by a 2-nitroketene, with an initial focus on the aromatic moiety (compounds **7**–**18**). These compounds were prepared by sequential displacement of the two methylthio groups of 1,1-bismethylthio-2-nitroethene with relevant amines.[14]

As shown in Table 1, replacement of the thiourea group in **1** with a 2-nitroketene aminal gives compound **7** with an IC_{50} of 6400 nM, which is 58-fold less active than **1**.

Table 1. SAR of the aromatic binding element



7-18

Compound	Ar	IC_{50} (nM)[a]
7	3-Me-Ph	6400
8	Ph	16500
9	3-MeO-Ph	6700
10	3-Me_2N-Ph	2100
11	4-MeO-Ph	3100
12	4-Me-Ph	>34000
13	4-Me_2N-Ph	>34000
14	3-Me-4-MeO-Ph	3400
15	2,3-Dihydro-benzofuran-5-yl	1500
16	Naphthalene-2-yl	1000
17	Benzofuran-5-yl	539
18	2-Methyl-benzofuran-5-yl	51

[a] IC_{50} are measured against human factor Xa utilizing the cleavage of a synthetic substrate S-2222. Data are the average of two independent determinations.

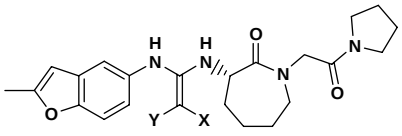
However, this compound is about 3-fold more potent than **8**, a compound without a *meta*-substitution on the phenyl ring. SAR studies indicate that other small *meta*-substituted phenyl derivatives are of comparable potency to **7** (for example, compounds **9** and **10**), while some *para*-substituents, with the exception of a methoxy, result in the loss of activity (compare compounds **11**, **12**, and **13**). The 3-methyl-4-methoxy substituted phenyl compound **14** has an IC_{50} similar to that of **11**.

Replacement of the phenyl ring in **8** with a 2-naphthyl group gives **16**, which is 16-fold more potent, indicating that a bicyclic moiety in this region may be a better pharmacophore. Compound **15**, which is a bicyclic analog of **14**, provides modest increase in potency. The 5-benzofuranyl group (**17**) results in a further increase in the potency. Finally, the 5-(2-methylbenzofuranyl) derivative provides the most potent compound in this series (**18**), with an IC_{50} of 51 nM and an $EC_{2\times PT}$[15] of 50 μM.

Similar SAR (data not shown) is observed when 2,2-dicyanoketene aminal is employed as the thiourea surrogate. Compound **19** (see Table 2) is about 2-fold more potent than its direct analog of nitroketene aminal **18**, with an IC_{50} of 30 nM and $EC_{2\times PT}$ of 19 μM.

The proposed binding mode of the ketene aminal compounds with FXa is illustrated with compound **19** in Figure 1.[16] The ketene aminal group provides the L-shaped scaffold seen in many FXa inhibitors, allowing the 2-methylbenzofuran group to fit deep in the S1 pocket and the acylpyrrolidine group to reside in S4. One of the nitrile groups is in close contact with the disulfide bond; the high degree of polarizability of both these groups could provide a strong favorable van der Waals component to the free energy of binding. The other

Table 2. SAR of the ketene substituents Scheme 3



Compound	X	Y	IC_{50} (nM)[a]	$EC_{2\times PT}$ (μM)[b]
19	CN	CN	30	19
27	CN	$CONH_2$	17	13
28	CN	CO_2Me	20	23
29	CN	CO_2Et	24	31
30	CN	CO_2t-Bu	57	60
31	CN	$CO_2(CH_2)_4OH$	10	10
32	CN	CO-4-methylpiperidine	57	21
33	CN	SO_2Me	46	24
34	CN	SO_2-*i*-Pr	647	nd
35	CN	CO-4-CI-Ph	13	62
36	CN	CO-4-MeO-Ph	13	36
37	CN	$CONHCONH_2$	13	7
38	CN	$PO(OEt)_2$	658	>60
39	H	CO-2-thiazole	10	31

[a] IC_{50} are measured against human factor Xa utilizing the cleavage of a synthetic substrate S-2222.
[b] Concentration of inhibitor required to double the prothrombin based clotting time in human plasma; data are the average of two independent determinations.

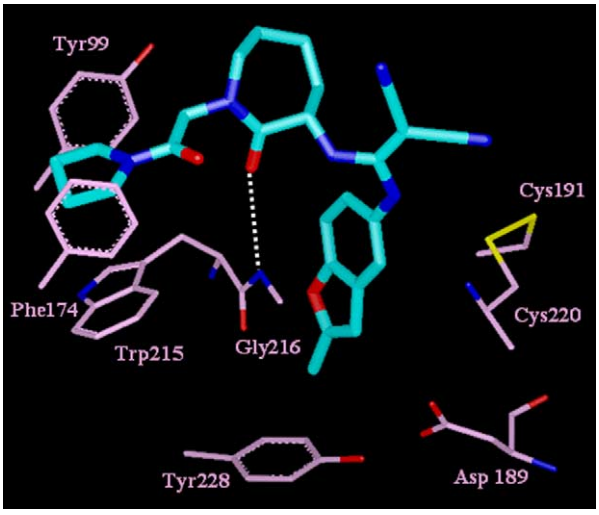


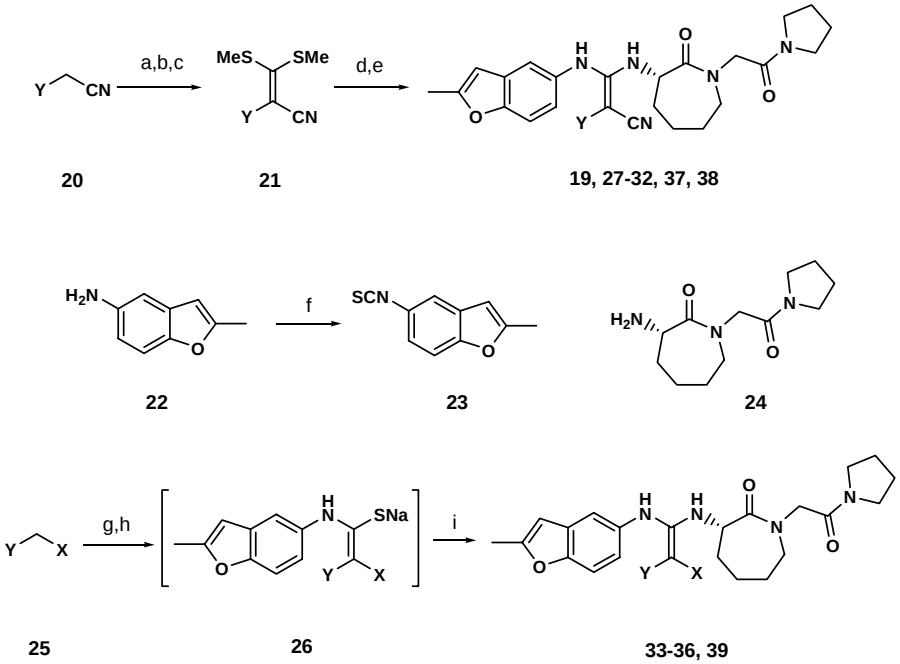
Figure 1. Model of **19** (cyan) in FXa (magenta). For clarity, only key residues are shown.

nitrile lies against the caprolactam ring and points toward solvent. Another key feature of this proposed binding mode is a hydrogen bond formed between the caprolactam carbonyl and the backbone NH of glycine 216 (3.3 Å in the model). From this model, it is clear that the methyl group could provide a key van der Waals interaction with Tyr228 and enhance the binding activity, which is reflected in the 10-fold increase in IC_{50} for compound **18** compared to **17**; it also could be proposed that variations of the ketene substitution groups may provide compounds with improved physiochemical properties without strongly impacting their anti-FXa activity.

To further explore the SAR of ketene substituents, additional compounds based on 5-(2-methylbenzofuranyl) as the S1 pharmacophore were synthesized according to Scheme 3. Compounds **19**, **27**–**32**, **37**, and **38** were synthesized by the stepwise displacement of both methylthio groups of ketene dithioacetals **21**, which was prepared by the reaction of the anion of **20** with carbon disulfide and then methylation of both thio-groups.[17] Compounds **33**–**36**, **39** were prepared according to a one-pot procedure we developed for *N*,*N*′-disubstituted ketene aminals.[12] For example, treatment of 2-(methylsulfonyl)acetonitrile (X = CN, Y = SO_2Me in **25**) with sodium hydride and reaction of the resulting anion with 2-methylbenzofuran-5-isothiocyanate **23** affords an intermediate thioamide anion (**26**). Subsequent reaction of **26** with caprolactam amine **24** and EDCI (1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride) provides **32**.

As shown in Table 2, compounds with differently substituted ketene aminals generally have similar FXa inhibitory activities to **19**, except for compounds **34** and **38**. The SAR is consistent with our binding model shown in Figure 1, where the ketene aminal adopts an *anti-syn* conformation with one ketene substituent pointing into the solvent. Modification of the ketene substituents leads to only slight improvement in anticoagulation activities ($EC_{2\times PT}$).

Similar to the lead compound **1**,[4] the ketene aminals described above are selective FXa inhibitors relative to related trypsin-like serine proteases. Table 3 shows the selectivity profiles of compound **31**. Furthermore, compound **31** showed activity in rats after systemic administration and measurement of ex vivo clotting time.[18] As



Scheme 3. The synthesis of ketene aminals **19**, **27**–**39**. Reagents and conditions: (a) KOH, H_2O, rt; (b) CS_2; (c) Me_2SO_4; (d) **22**, EtOH, 70 °C; (e) **24**, EtOH, 70 °C; (f) 1,1′-thiocarbonyldi-2(1*H*)-pyridone, CH_2Cl_2; (g) NaH, DMF; (h) **23**, 60 °C; (i) **24**, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride.

Table 3. Selectivity profile of **31**

Human enzymes	K_i (nM)[a]
Factor Xa	12
Trypsin	>5000
Thrombin	6300
Plasma kallikrein	>19400
Activated protein C	>21500
Factor IXa	>10000
Factor VIIa	>55000
Chymotrypsin	9420
Urokinase	>14000
Plasmin	>22000
tPA	16000

[a] K_i's are calculated from the IC_{50} values assuming competitive inhibition versus the low molecular weight synthetic substrates using the relationship, $K_i = IC_{50}/(1 + [S]/K_m)$, where [S] is the substrate concentration in the assay and K_m is the Michaelis constant for that substrate. Data are the average of two independent determinations.

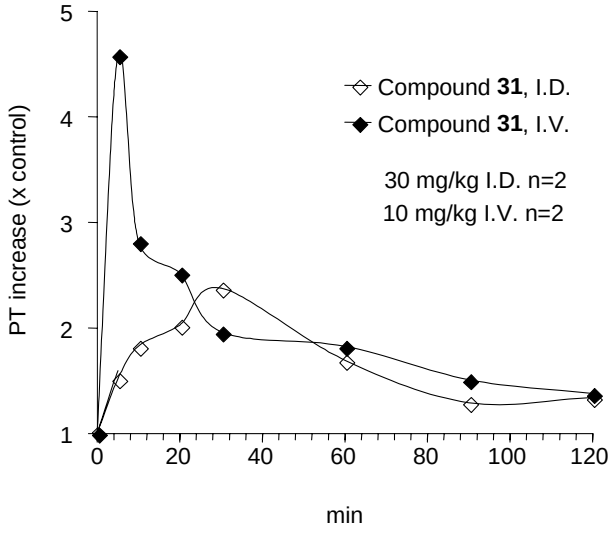


Figure 2. The iv and id dosing of **31** in rat.

shown in Figure 2, intravenous bolus injection of 10 mg/kg of **31** led to a 1.38-fold increase in prothrombin time (PT) after 2 h, and intraduodenal dosing of 30 mg/kg of **31** resulted in a 1.35-fold increase in PT after 2 h. Compound **31** also partially protected against lethality in mice induced by intravenous injection of Russel's viper venom (which causes FXa activation and lethality due to pulmonary thrombosis). Vehicle-treated mice died within 1.0 ± 0.1 min ($n = 16$) after Russel's viper venom injection, while survival times were increased to 16.0 ± 8.4 min ($n = 5$) after oral administration of 2 mg/kg of **31**.[19]

In summary, we have discovered a series of novel, orally active FXa inhibitors. These compounds exhibit potent and highly selective anti-FXa activity. The SAR studies led to the 2-methylbenzofuran as the critical S1 binding element, and that the ketene aminals are good bioisosteres for the thiourea functional group.

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15. IC_{50} are measured against human factor Xa utilizing the cleavage of a synthetic substrate S-2222. The $EC_{2\times PT}$ is the concentration of inhibitor required to double the prothrombin based clotting time in human plasma.
16. Initial binding mode was obtained based on an in-house FXa crystal structure of a compound identical to **19** but with an acylguanidine linker in place of the ketene aminal. From this starting point, the compound was minimized in the published FXa crystal structure 1fjs (Adler, M.; Davey, D. D.; Phillips, G. B.; Kim, S. H.; Jancarik, J.; Rumennik, G.; Light, D. L.; Whitlow, M. *Biochemistry* **2000**, *39*, 12534. 1.92 Å resolution) using Discover v. 98.0 in the InsightII package (Accelrys, Inc.) with the CFF forcefield. All residues in FXa were fixed with the exception of Tyr99, Phe174, Cys191, Cys220, Trp215, Ser195, Gln192, Arg143, Glu146, and Gly218.
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19. Swiss Webster mice (24–38 g) were anesthetized with sodium pentobarbital (100 mg/kg, ip). Russell's viper venom (Sigma Chemical, St. Louis, MO) was prepared in 0.9% saline and injected into the tail vein at a dose of 7 μg/mouse in a volume of 0.1 mL given 15 min after induction of anesthesia. This dose of venom was found to be uniformly fatal in 105 mice with death occurring in an average of 1.5 ± 1.9 min (±SD, range was 1 to 12 min with 88% of mice dying within 2 min). Histological evaluation of several venom-treated mice revealed that early death was due to occlusion of pulmonary capillaries with fibrin aggregates, probably caused by initiation of blood coagulation by venom activation of factor X to factor Xa. A 2 mg/kg dose of test compounds or saline vehicle was administered by oral gavage 2 h before the venom injection. Survival was monitored out to 30 min after venom injection.