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Amino(methyl) pyrrolidines as novel scaffolds for factor Xa inhibitors

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Dedicated to the memory of Karnail S. Atwal.

Abstract—The design and synthesis of a novel class of amino(methyl) pyrrolidine-based sulfonamides as potent and selective FXa inhibitors is reported. The amino(methyl) pyrrolidine scaffolds were designed based on the proposed bioisosterism to the piperazine core in known FXa inhibitors. The SAR study led to compound **15** as the most potent FXa inhibitor in this series, with an IC_{50} of 5.5 nM and PT EC_{2x} of 1.7 μM. The proposed binding models show that the pyrrolidine cores are in van der Waals contact with the enzyme surface, and the flexibility of amino(methyl) pyrrolidines allows the two nitrogen atoms to anchor both the P1 and P4 groups to fit similarly in the S1 and S4 pockets.

Factor Xa is one of the targets for the design of new antithrombotics.[1] Selective factor Xa inhibitors may effectively block coagulation, since factor Xa is positioned at the start of the common pathway of the extrinsic and intrinsic coagulation systems.[2] It has been hypothesized that, because the amount of serine protease is amplified at each step of the coagulation cascade, anticoagulants which target coagulation factors located higher up in the cascade, such as factor Xa, might be more effective than those directly targeting thrombin. Moreover, by not inhibiting thrombin directly, residual thrombin may still facilitate hemostasis and reduce unwanted bleeding risk.[2c] Clinical studies with the parenteral, indirect factor Xa inhibitor fondaparinux indicate that selective inhibition of factor Xa is a highly effective approach to the prevention and treatment of venous thromboembolism.[3] Recent clinical trial disclosures on oral, direct factor Xa inhibitors such as razaxaban,[4a–d] apixaban,[4e] rivaroxaban[4f,g], and LY717717[4h,i] have provided clinical evidence of preclinical findings.[4]

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] Several series of potent FXa inhibitors containing the 1,4-substituted piperazine or 2-oxo-piperazine cores have been reported.[5] Among them, compound **1** (Fig. 1) was first disclosed in a Zeneca patent application in 1996,[5a] and was subsequently reported to have an IC_{50} of 210 nM against human FXa by researchers from Mochida.[5b] Compound **2** (Fig. 1) was described to inhibit FXa with an IC_{50} of 19 nM in a Takeda patent application in 1998.[5c] More recently, several X-ray crystal structures of structurally related molecules bound to FXa have been disclosed.[5d–f] They show that the two nitrogen atoms of the piperazine or 2-oxopiperazine core are positioned near the entry points of the S1 and S4 pockets, and the 'bent' conformation of the molecule is created by appropriate substitutions at these two nitrogen atoms. We envisioned that the 3-aminomethyl-pyrrolidine could be a potential bioisostere for the piperazine core due to the relatively planar conformation of the pyrrolidine ring in combination with the flexible

Keywords: Aminomethyl pyrrolidines; Factor Xa inhibitor; Scaffold; Bioisosterism; Piperazine.
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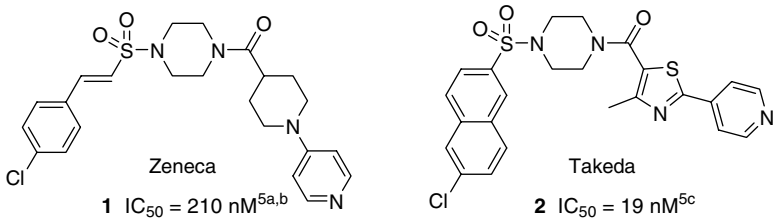


Figure 1. Substituted piperazine-based inhibitors of human factor Xa **1** and **2**.

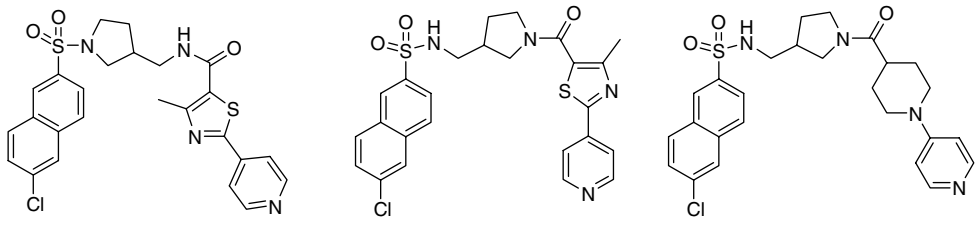


Figure 2. 3-Aminomethyl-pyrrolidine-based inhibitors of human factor Xa **3**–**5**.

3-aminomethyl group. To test this hypothesis, we synthesized three racemic compounds **3**, **4**, and **5** (Fig. 2), which combine the new 3-aminomethyl-pyrrolidine core with known sulfonamide and amide moieties in **1** and **2**. While both **3** and **4** showed weak activity against human FXa, a clear preference for the 3-arylsulfonylaminomethyl-1-acylpyrrolidine **4** was observed. The exchange of the thiazole-pyridine moiety in **4** with a 1-(4-pyridyl)piperidine group provided the more potent analog **5**, which has an IC_{50} of 247 nM[6] against human FXa. Based on this lead, we initiated SAR studies to further improve the potency. This communication describes the in vitro SAR of this novel series of aminomethylpyrrolidine-based FXa inhibitors.[7]

Early efforts in this study focused on analogs of **5** where the arylsulfonyl group was varied. Selected analogs are shown in Table 1 to illustrate the SAR. These compounds were prepared according to Scheme 1. Acylation of the pyrrolidine nitrogen in **6** with 1-(pyridin-4-yl)piperidine-4-carboxylic acid **7** affords **8**. Subsequent deprotection of the Boc group and reaction with appropriate arylsulfonyl chlorides provides **5** (65% yield) and **9**–**17** (15–73% yield).

As shown in Table 1, the unsubstituted 2-naphthyl compound **9** is about 5-fold less potent than the 6-chloronaphthyl analog **5**, which indicates the importance of the chlorine atom. Replacement of the 6-chloro-2-naphthyl group in **5** with a (*E*)-3-chlorostyryl group provides **10**, which is about 20-fold less active. However, the regioisomeric (*E*)-4-chlorostyryl compound **11** (racemate) is only about 2-fold less potent than **5**, and is about 2-fold less potent than the piperazine derivative **1** (210 nM, Fig. 1) with the same pharmacophores at both ends. This indicates a trans olefin-aryl group may be a viable pharmacophore in this region. The 6-chloro-2-yl-benzo[*b*]thiophene compound **12** has similar FXa activity to **5**.

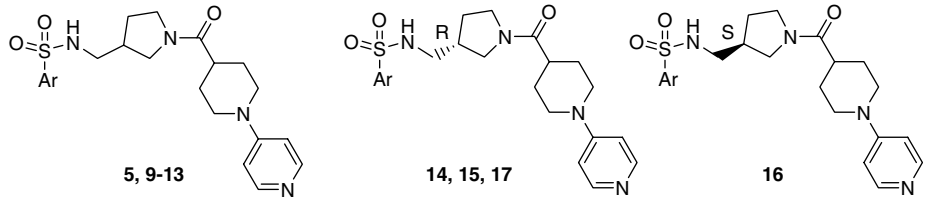
Replacement of the 4-chlorophenyl group in **11** with a 5-bromo-2-yl-thiophene moiety affords the more potent analog **13** (IC_{50} = 86 nM). Its (*R*)-enantiomer, compound **14**, appears to be the more active stereoisomer with an IC_{50} of 22 nM. The (*E*)-2-(5-chlorothiophen-2-yl)ethene derivatives (compounds **15** and **16**) further demonstrate the preference for the (*R*)-enantiomer **15**. The (*R*)-enantiomer **15** has an IC_{50} of 5.5 nM (PT EC_{2x} = 1.7 μM),[6] and is about 25-fold more potent than its (*S*)-enantiomer **16** (IC_{50} = 150 nM). The 5′-chloro-2,2′-bithiophene moiety also provides potent compounds, for example, **17** (IC_{50} = 18 nM).

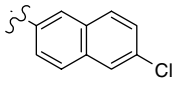
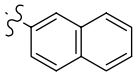
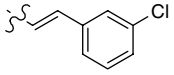
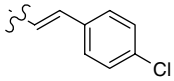
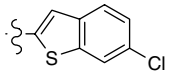
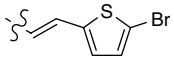
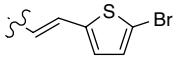
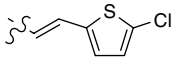
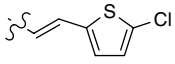
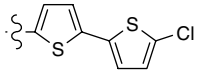
To explore the SAR of the amide moiety appended to the pyrrolidine ring nitrogen, additional compounds based on 5′-chloro-2,2′-bithiophene-5-yl- and (*E*)-2-(5-chlorothiophen-2-yl)ethane-sulfonamide were synthesized according to Scheme 2. Selected analogs are shown in Table 2 to illustrate the SAR. These compounds were prepared by formation of sulfonamide **19** from optically pure *tert*-butyl 3-(aminomethyl)pyrrolidine-1-carboxylate **18** and appropriate sulfonyl chlorides; subsequent Boc deprotection of **19** and amide formation reaction with a diverse set of carboxylic acids affords **20**–**28**.

As shown in Table 2, compounds with a biaryl amide substrate are generally disfavored in both *R* and *S* series (**20** and **21**). The 2-methylpyrimidine compounds are less potent than their pyridine analogs (compare compounds **24**–**26** with **15**–**17**). Interestingly, some substituted oxalic pyrrolidines in the S series exhibit potent FXa inhibition activities (compounds **23**, **27**, and **28**), with compound **28** having an IC_{50} = 40 nM and PT EC_{2x} = 13 μM.

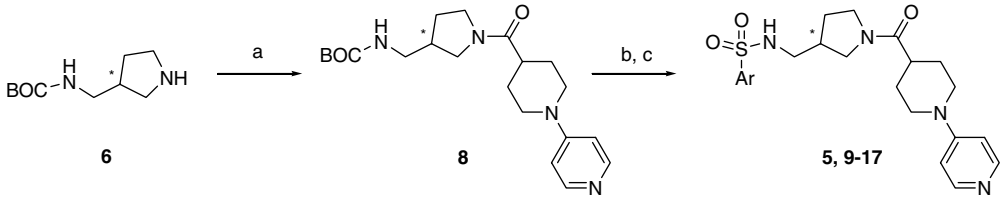
The proposed binding models of compounds **17** and **28** with FXa are shown in Figures 3 and 4,

Table 1. SAR of the aromatic binding element



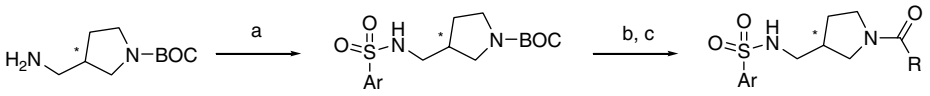
Compound	Ar	Chirality	IC_{50}[a] (nM)	PT EC_{2x}[b] (μM)
5		(±)	247	40
9		(±)	1100	—
10		(±)	4300	—
11		(±)	493	32
12		(±)	255	45
13		(±)	86	8.7
14		*R*	22	5
15		*R*	5.5	1.7
16		*S*	150	13
17		*R*	18	6.8

[a] IC_{50} values 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.



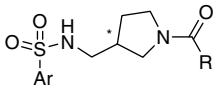
Scheme 1. The synthesis of **5**, **9**–**17**. Reagents: (a) 1-(pyridin-4-yl)piperidine-4-carboxylic acid (**7**), EDCI, Et_3N, CH_2Cl_2; (b) TFA, CH_2Cl_2; (c) arylsulfonyl chloride, EtOAc/$NaHCO_3$ (satd), 15–73% yield for three steps.

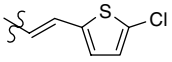
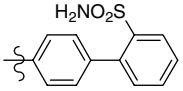
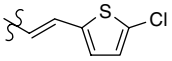
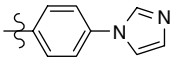
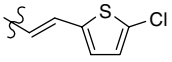
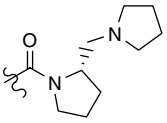
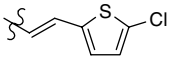
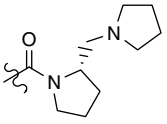
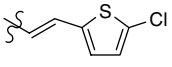
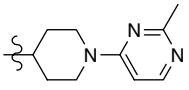
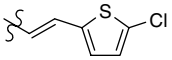
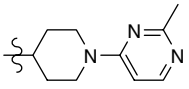
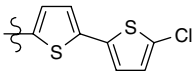
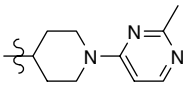
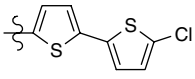
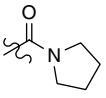
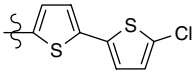
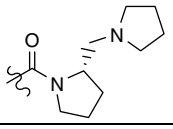
respectively.[8] In both models, the aminomethyl pyrrolidine cores lie along the surface of the protein. The two nitrogen atoms of the aminomethyl pyrrolidine group provide the anchor points for the P1 and P4 moieties. In the P1 direction, the arylsulfonyl group creates the needed L-shaped turn seen in many FXa inhibitors, allowing the chlorothiophene group to fit deep into the S1 pocket where the chlorine atom fills a key hydrophobic interaction site above Tyr228. In the S4 pocket, the protonated 4-(piperidin-1-yl)pyridine group of **17** participates in a hydrogen bond with a conserved water molecule, and forms a π–cation



Scheme 2. The synthesis of **20**–**28**. Reagents: (a) arylsulfonyl chloride, EtOAc/$NaHCO_3$ (satd); (b) TFA, CH_2Cl_2; (c) RCOOH, EDCI, Et_3N, CH_2Cl_2, 10–71% yield over three steps.

Table 2. SAR of the amide pharmacophore



Compound	Ar	R	Chirality	IC_{50}[a] (nM)	PT EC_{2x}[b] (μM)
20			*R*	1900	—
21			*S*	1300	—
22			*R*	436	—
23			*S*	70	8
24			*R*	230	28
25			*S*	718	—
26			*R*	61	50
27			*S*	130	130
28			*S*	40	13

[a] IC_{50} values 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.

interaction with the aromatic residues lining the pocket. For compound **28**, the 2-pyrrolidinomethylpyrrolidine fills the S4 pocket with the distal pyrrolidine forming an intramolecular hydrogen bond with the amide carbonyl seen in many κ Opioid agonists[9]; the presence of this additional pyrrolidine enhances the interaction between the acyl pyrrolidine and S4 pocket (compare compounds **27** and **28**). In addition, the potential hydrogen bonding interaction between Gln192 side chain and one of the sulfonamide oxygens in both models may also contribute to the binding activity. Docking studies of **15** versus **16** suggest the activity difference (25-fold) may be due to the energetics of the different conformations of the aminomethyl

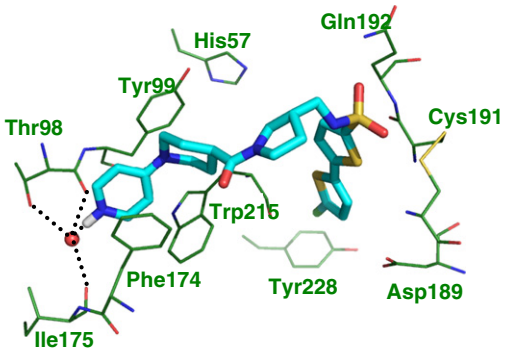


Figure 3. Model of **17** bound to FXa.

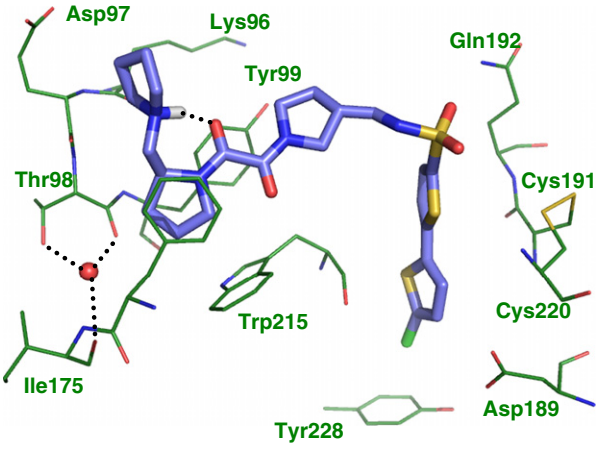


Figure 4. Model of **28** bound to FXa.

substituent relative to the pyrrolidine core—the *R* conformation is equatorial, whereas the *S* conformation is axial and must fold back on the pyrrolidine to dock well.

From these two models, it is clear that the center pyrrolidine cores function as scaffolds, where the primary interaction with the enzyme is through van der Waals contacts on the bottom face of the core, similar to the role played by the cores seen in crystal structures of piperazine-based ligands in FXa.[5e,f] The flexibility of the aminomethyl pyrrolidine group allows both enantiomeric cores to project the P1 and P4 pharmacophores into the S1 and S4 pockets. It could be proposed that 2-aminomethyl pyrrolidine and 3-aminopyrrolidine with a two-carbon spacer between two nitrogens may also be good scaffolds for FXa inhibitors. Indeed, compounds such as racemate **29** and **30** (Fig. 5), with the same P1 and P4 pharmacophores as in **17**, are also potent FXa inhibitors with FXa IC_{50} of 5.5 nM and 7.9 nM, respectively. Figure 6 shows the overlay of models of **17**, **29** (*S* enantiomer), and **30** (*S* enantiomer) bound to FXa, which demonstrates that the three central core variants enable the P1 and P4 groups to fit similarly in the S1 and S4 pockets.

Although the preferred absolute stereochemistry for **29** and **30** has not been determined, similar SAR in the P1 binding elements have been observed for both the 3-aminomethyl pyrrolidine and 3-aminopyrrolidine series when the P4 pharmacophore is a 1-(4-pyridyl)-piperidine group (data not shown).

While the compounds described above are potent and selective FXa inhibitors relative to related trypsin-like serine proteases,[10] compounds containing a 1-(4-pyridyl)piperidine group as P4 pharmacophore are generally moderate inhibitors of cytochrome P450 and have limited Caco-2 cell permeability.[11] Further work would be necessary to address these issues before this series can furnish acceptable antithrombotic drug candidates.

In summary, we have discovered a novel series of FXa inhibitors. These compounds exhibit potent and highly selective anti-FXa activity. The SAR demonstrates that both 2- and 3-aminomethyl pyrrolidines and 3-aminopyrrolidine are excellent bioisosteres for the piperazine core in FXa inhibitors with similar P1 and P4 pharmacophores. The SAR is consistent with binding models which show that the pyrrolidine cores are in van der Waals contact with the enzyme surface, and the flexibility of amino(methyl) pyrrolidines allows the two nitrogen atoms to anchor both the P1 and P4 groups to fit similarly in the S1 and S4 pockets.

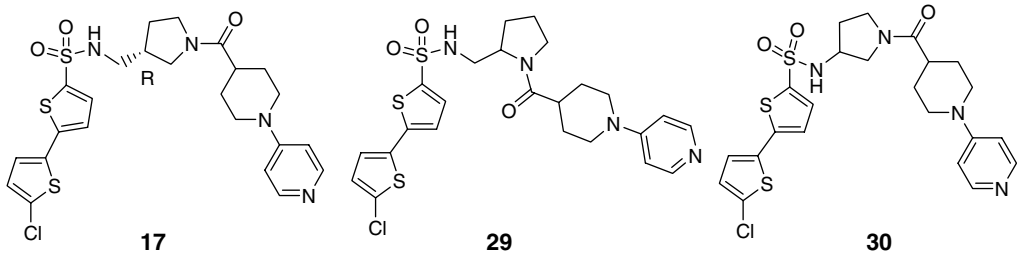


Figure 5. The SAR of amino(methyl)pyrrolidines.

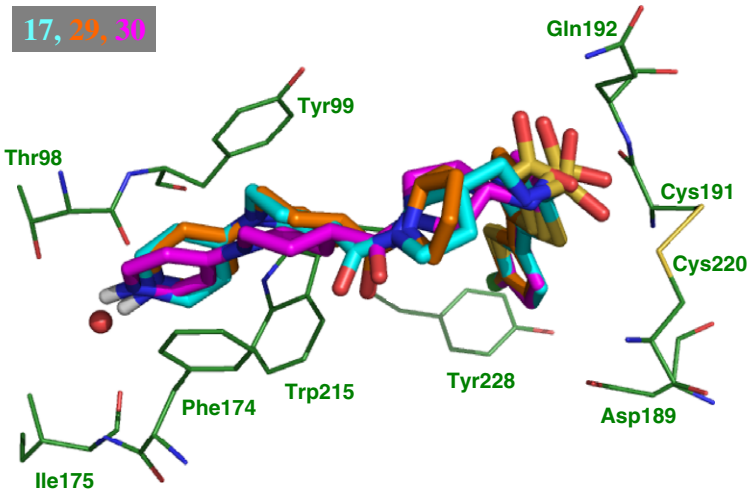


Figure 6. The overlay of models of **17**, **29**, and **30** (*S* enantiomers) bound to FXa.

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References and notes

1. For reviews, see: (a) Kunitada, S.; Nagahara, T.; Hara, T. *Handbook Exp. Pharmacol.* **1998**, 397; (b) Vacca, J. P. *Ann. Rep. Med. Chem.* **1998**, *33*, 81; (c) Zhu, B.-Y.; Scarborough, R. M. *Curr. Opin. Cardiov. Pulm. Ren. Investig. Drugs* **1999**, *1*, 63; (d) Ewing, W. R.; Pauls, H. W.; Spada, A. P. *Drugs Future* **1999**, *24*, 771; (e) Fevig, J. M.; Wexler, R. R. *Ann. Rep. Med. Chem.* **1999**, *34*, 81; (f) Betz, A. *Expert Opin. Ther. Patents* **2001**, *11*, 1007.
2. (a) Davie, E. W.; Fujikawa, K.; Kisiel, W. *Biochemistry* **1991**, *30*, 10363; (b) Butenas, S.; Van't Veer, C.; Cawthern, K.; Brummel, K. E.; Mann, K. G. *Blood Coagul. Fibrinolysis* **2000**, *11*(Suppl. 1), S9; (c) Wong, P. C.; Watson, C. A.; Crain, E. J.; Ogletree, M. L.; Wexler, R. R.; Lam, P. Y. S.; Pinto, D. J.; Knabb, R. M. *J. Thromb. Haemost.* **2007**, *5*(Suppl. 1), Abstr. 1315.
3. (a) Turpie, A. G.; Eriksson, B. I.; Lassen, M. R.; Bauer, K. A. *J. South. Orthop. Assoc.* **2002**, *11*, 182; (b) Turpie, A. G.; Bauer, K. A.; Eriksson, B. I., et al. *Arch. Intern. Med.* **2002**, *162*, 1833; (c) Eriksson, B. I.; Lassen, M. R. *Arch. Intern. Med.* **2003**, *163*, 1337; (d) Hobbelen, P. M.; van Dinther, T. G.; Vogel, G. M. T.; van Boeckel, C. A. A.; Moelker, H. C. T.; Meuleman, D. G. *Thromb. Haemost.* **1990**, *63*, 265.
4. (a) Quan, M. L.; Lam, P. Y. S.; Han, Q.; Pinto, D. J. P.; He, M. Y.; Li, R.; Ellis, C. D.; Clark, C. G.; Teleha, C. A.; Sun, J.-H.; Alexander, R. S.; Bai, S.; Luettgen, J. M.; Knabb, R. M.; Wong, P. C.; Wexler, R. R. *J. Med. Chem.* **2005**, *48*, 1729; (b) Pinto, D. J. P.; Orwat, M. J.; Wang, S.; Fevig, J. M.; Quan, M. L.; Amparo, E.; Cacciola, J.; Rossi, K. A.; Alexander, R. S.; Smallwood, A. M.; Luettgen, J. M.; Liang, L.; Aungst, B. J.; Wright, M. R.; Knabb, R. M.; Wong, P. C.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2001**, *44*, 566; (c) Lam, P. Y. S.; Clark, C. G.; Li, R.; Pinto, D. J.; Orwat, M. J.; Galemmo, R. A.; Fevig, J. M.; Teleha, C. A.; Alexander, R. A.; Smallwood, A. M.; Rossi, K. A.; Wright, M. R.; Bai, S. A.; He, K.; Luettgen, J. M.; Wong, P. C.; Knabb, R. M.; Wexler, R. R. *J. Med. Chem.* **2003**, *46*, 4405; (d) Lassen, M. R.; Davidson, B. L.; Gallus, A.; Pineo, G.; Ansell, J.; Deitchman, D. *Blood* **2003**, *102*, Abstr. 41; (e) Pinto, D. J. P.; Orwat, M. J.; Koch, S.; Rossi, K. A.; Alexander, R. S.; Smallwood, A. M.; Wong, P. C.; Rendina, A.; Luettgen, J. M.; Knabb, R. M.; He, K.; Xin, B.; Wexler, R. R.; Lam P. Y. S. *J. Med. Chem.*, in press; (f) Straub, A.; Pohlmann, J.; Lampe, T.; Pernerstorfer, J.; Schlemmer, K.-H.; Reinemer, P.; Perzborn, E.; Roehrig, S. *J. Med. Chem.* **2005**, *48*, 5900; (g) Eriksson, B. L.; Borris, L.; Dahl, O. E.; Haas, S.; Huisman, M. V.; Kakkar, A. K. *J. Thromb. Haemost.* **2006**, *4*, 121; (h) Liebeschuetz, J. W.; Jones, S. D.; Wiley, M. E.; Young, S. C. *Struct.-Based Drug Discovery* **2006**, 173; (i) Hampton, T. *JAMA* **2006**, *295*, 743; (j) Eriksson, B. I.; Quinlan, D. J. *Drugs* **2006**, *66*, 1141; (k) Saiah, E.; Soares, C. *Curr. Top. Med. Chem.* **2005**, *5*, 1677.
5. (a) Faull, A. W.; Mayo, C. M.; Preston, J. Int. Pub. No. WO 96/10022, April 4, 1996; (b) Nishida, H.; Miyazaki, Y.; Kitamura, Y.; Ohashi, M.; Matsusue, T.; Okamoto, A.; Hosaka, Y.; Ohnishi, S.; Mochizuki, H. *Chem. Pharm. Bull.* **2001**, *49*, 1237; (c) Tawada, H.; Ito, F.; Moriya, N.; Terashita, Z. Int. Pub. No. WO 98/54164, December 3, 1998; (d) Maignan, S.; Guilloteau, J.-P.; Choi-Sledeski, Y. M.; Becker, M. R.; Ewing, W. R.; Pauls, H. W.; Spada, A. P.; Mikol, V. *J. Med. Chem.* **2003**, *46*, 685; (e) Haginoya, N.; Kokayashi, S.; Komoriya, S.; Yoshino, T.; Suzuki, M.; Shimada, T.; Watanabe, K.; Hirokawa, Y.; Furugori, T.; Nagahara, T. *J. Med. Chem.* **2004**, *47*, 5167; (f) Komoriya, S.; Haginoya, N.; Kokayashi, S.; Nagata, T.; Mochizuki, A.; Suzuki, M.; Yoshino, T.; Horino, H.; Nagahara, T.; Suzuki, M.; Isobe, Y.; Furugoori, T. *Bioorg. Med. Chem.* **2005**, *13*, 3927; (g) Matsusue, T.; Shiromizu, I.; Nishida, H.; Mukaihira, T.; Saitoh, F.; Hosaka, Y.; Morishita, H.; Mochizuki, H.; Ohnishi, S. *Abstr. Pap. Am. Chem. Soc* **2004**, *228*, MEDI-255; (h) Nishida, H.; Miyazaki, Y.; Mukaihira, T.; Saitoh, F.; Fukui, M.; Harada, K.; Itoh, M.; Muraoka, A.; Matsusue, T.; Okamoto, A.; Hosaka, Y.; Matsumoto, M.; Ohnishi, S.; Mochizuki, H. *Chem. Pharm. Bull.* **2002**, *50*, 1187; (i) Nishida, H.; Mukaihira, T.; Saitoh, F.; Harada, K.; Fukui, M.; Matsusue, T.; Okamoto, A.; Hosaka, Y.; Matsumoto, M.; Shiromizu, I.; Ohnishi, S.; Mochizuki, H. *Chem. Pharm. Bull.* **2004**, *52*, 406; (j) Haginoya, N.; Kokayashi, S.; Komoriya, S.; Yoshino, T.; Nagata, T.; Hirokawa, Y.; Nagahara, T.

Bioorg. Med. Chem. **2004**, *12*, 5579; (k) Haginoya, N.; Kokayashi, S.; Komoriya, S.; Hirokawa, Y.; Furugori, T.; Nagahara, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2935; (l) Jia, Z. J.; Su, T.; Zuckett, J. F.; Wu, Y.; Goldman, E. A.; Li, W.; Zhang, P.; Clizbe, L. A.; Song, Y.; Bauer, S. M.; Huang, W.; Woolfrey, J.; Sinha, U.; Arfsten, A. E.; Hutchaleelaha, A.; Hollenbach, S. J.; Lambing, J. L.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2073.

6. IC_{50}s are measured against human factor Xa utilizing the cleavage of a synthetic substrate S-2222. The PT EC_{2x} is the concentration of inhibitor required to double the prothrombin-based clotting time in human plasma. See: Kettner, C. A.; Mersinger, L. J.; Knabb, R. M. *J. Biol. Chem.* **1990**, *265*, 18289.
7. This work was presented in part by: Shi, Y.; Zhang, J.; Stein, P. D.; Wang, Y.; Kennedy, L.; O'Connor, S. P.; Ahmad, S.; Atwal, K. S.; Han, W.; Hu, Z.; Sitkoff, D.; Lam, P. Y. S.; Robl, J. A.; Liu, E. C.-K.; Seiler, S. M.; Hartl, K. S.; Wexler, R. R.; Zahler, R. *Abstr. Pap. Am. Chem. Soc.* **2006**, *232*, MEDI-395.
8. Compounds were docked into the FXa enzyme structure from pdb entry 2bmg (Matter, H.; Will, D. W.; Nazare, M.; Schreuder, H.; Laux, V.; Wehner, V.; Liesum, A. *J. Med. Chem.* **2005**, *48*, 3290) using Glide (v. 4.0, Schrodinger, LLC, New York, NY, 2005). Prior to docking, all waters were removed except the water in the S4 pocket, and hydrogens were assigned using the prot-assign utility. The enzyme was then run through the Glide proteinprep and refine utilities. Docking was performed in sp mode with default parameters except that the grids for the enzyme active site were calculated using an 0.9 (instead of 1.0) scaling factor on the vdw radii of protein atoms with an absolute value of partial atomic charge less than 0.25 e^-. Resulting docked conformations within 1.5 U of the best glidescore were qualitatively similar; a single example was selected by eye for inclusion in the figures. For **28**, the structures from Glide had no internal hydrogen bond, so the conformation was altered based on known preferred conformations of κ Opioid agonists, see Ref. 9.
9. At physiologic pH (7.2–7.4), the distal pyrrolidine N (ACD $pK_a = 9.69$) is protonated and forms an intramolecular hydrogen bond with the amide carbonyl. This preferred conformation of similar moieties which contain a *N*-(2-aminoethyl)acetamide had been intensively investigated in many κ Opioid agonists by molecular modeling, NMR studies, and X-ray analyses. See: (a) Froimowitz, M.; Dimeglio, C. M.; Makriyannis, A. J. *J. Med. Chem.* **1992**, *35*, 3087; (b) Subramanian, G.; Paterlini, M. G.; Larson, D. L.; Portoghese, P. S.; Ferguson, D. M. *J. Med. Chem.* **1998**, *41*, 4777; (c) Vecchietti, V.; Giordani, A.; Giardina, G.; Colle, R.; Clarke, G. D. *J. Med. Chem.* **1991**, *34*, 397; (d) Doi, M.; Ishida, T.; Inoue, M. *Acta Crystallogr.* **1990**, *C46*, 676; (e) Doi, M.; Ishida, T.; Inoue, M. *Chem. Pharm. Bull.* **1990**, *38*, 1815; (f) Chang, A.-C.; Takemori, A. E.; Ojala, W. H.; Gleason, W. B.; Portoghese, P. S. *J. Med. Chem.* **1994**, *37*, 4490.
10. For example, compound **17** is inactive ($IC_{50} > 30$ μM) when tested against thrombin, trypsin, Factor IXa, Factor VIIa, and plasma kallikrein.
11. For example, compound **17** has Caco-2 cell permeability (apical to basal) < 15 nm/s at pH 6.5; it inhibits CYP450 isozymes with the following IC_{50} values: 3A4 (7-benzyloxy-4-trifluoromethylcoumarin), 2.33 μM; 3A4 (7-benzyloxyresorufin), 1.0 μM; 2D6, 7.97 μM; 2C9, 1.47 μM; 2C19, 14.1 μM; 1A2, >20 μM.