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Investigation of the terminal P4 domain in a series of D-phenylglycinamide-based factor Xa inhibitors

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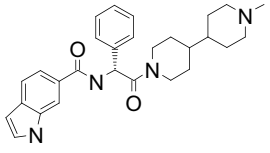
Abstract—Several P4 domain derivatives of the general D-phenylglycinamide-based scaffold (**2**) were synthesized and evaluated for their ability to bind to the serine protease factor Xa. Some of the more potent compounds were evaluated for their anticoagulant effects in vitro. A select subset containing various P1 indole constructs was further evaluated for their pharmacokinetic properties after oral administration to rats.

Factor Xa (fXa) is a serine protease that plays a crucial role in the blood coagulation cascade.[1] FXa occupies a common point in both the intrinsic and extrinsic pathways and is responsible for the production of thrombin. Thrombin, another serine protease, converts fibrinogen to fibrin, and fibrin cross-links with platelets eventually leading to blood clot formation. Because fXa is essential to this pathway, it has been hypothesized that the design of a small molecule inhibitor of factor Xa would be useful in treating patients with thromboembolic diseases.[2]

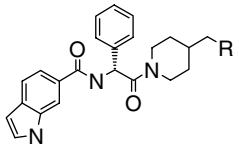
Previously, our laboratories reported the discovery of D-phenylglycine-based inhibitors of fXa (compound **1**).[3] SAR studies carried out in the P1 portion of these inhibitors led to the discovery that fused bicyclic elements enhanced binding affinity in vitro.[4] Compound **1**, which contains a 6-indolyl ring system, is a potent inhibitor of factor Xa (K_{ass}). In addition to having potent binding affinity for fXa, compound **1** also displays reasonable anticoagulant activity as measured by doubling prothrombin time in vitro (2× PT).[5]



1
Kass[a] = 450 x 10^6 L/mol
2x PT[b] = 1.5 μM

[a] Kass represents the apparent association constant, as measured by the methods of Smith[5] et al.
[b] 2x PT is defined as the concentration of compound required to double the time to clot formation in the prothrombin time assay.

A crystal structure of **1** (Fig. 1) indicates the 6-indolyl ring binds in the S1 pocket and the *N*-methylbispiperidine domain binds in the S4 region of this protease.



2

Keywords: Factor Xa inhibitors; D-Phenylgylcinamides; Antithrombotics.
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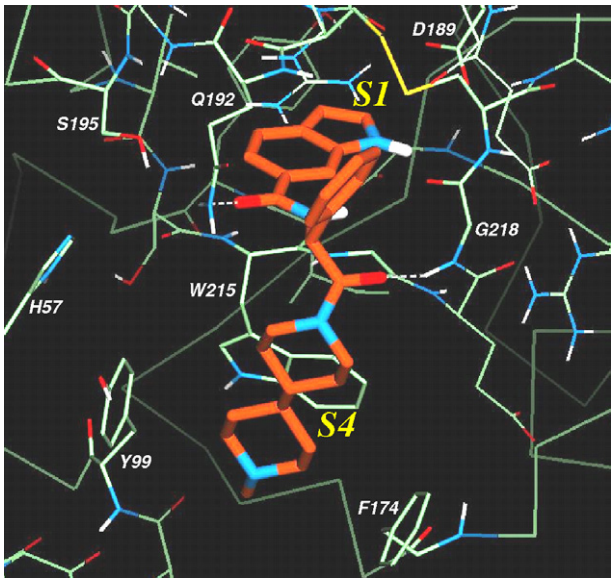
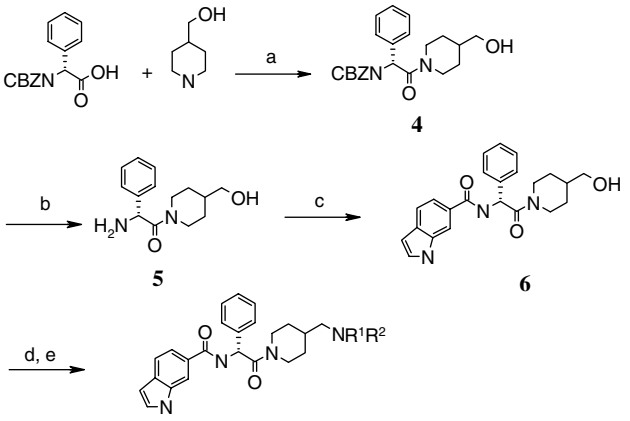


Figure 1. Factor Xa complex with compound **1**. X-ray crystal structure (1.90 Å resolution).

In this paper, we present the synthesis and terminal P4 domain SAR for a select set of compounds of formula **2** in which hydrogen bonding motifs are incorporated. The effect on anticoagulant activity in vitro of compounds displaying the highest binding affinity is presented. Additionally, a select subset of derivatives containing specific P1 fused bicyclic constructs and their binding affinity to fXa, anticoagulant potency, and oral exposure properties are reported.

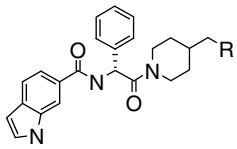
The synthetic approach utilized for compounds **3a**–**3l** is shown in Scheme 1. The commercially available CBZ protected D-phenylglycine is coupled with 4-piperidinemethanol to afford **4** in high yield with minimal epimerization. Removal of the CBZ group proceeds smoothly to provide the free amine **5**, and coupling with indole-6-carboxylic acid affords **6** in modest yield. Mesylation of **6** followed by displacement with a variety of amines results in the final compounds.



Scheme 1. Reagents and conditions: (a) EDCI, HOAt, DCM/DMF (1:1), 94%, >95% ee; (b) 10% Pd/C, H_2, EtOAc/EtOH (1:1), 94%; (c) DCC, HOBt, DMF, indole-6-carboxylic acid, 54%; (d) DCM, MsCl, NEt_3, 83%; (e) THF, NaI, K_2CO_3, NR^1R^2, 60 °C, 25–55% yield.

The factor Xa binding affinity and anticoagulant potency for compounds **3a**–**3l** are listed in Table 1. Compounds **3b** and **3c** with a tertiary amine have a higher binding affinity than the secondary amine **3a**. The pyrrolidine derivatives that contain a hydrophilic hydroxyl group (**3d**–**3g**) are less active than the unsubstituted pyrrolidine **3c**. Similarly, the piperidine derivatives containing a hydroxyl group **3i** and **3j** have lower binding affinity than the unsubstituted piperidine **3h**. The binding affinity for fXa is increased 2-fold when the ring size is expanded from 5 to 6 atoms (**3c** vs **3h**). Incorporation of a second nitrogen atom into the piperidine ring re-

Table 1. Human factor Xa binding affinity and anticoagulant activity of select inhibitors **3a**–**3l**



Compound	R	K_{ass}[a] (×10⁶ L/mol)	2× PT[b] (μM)
1	—	450	1.5
3a		7.8	—
3b		28	2.3
3c		24	2.2
3d		18	2.2
3e		9.7	—
3f	(rac) 	5.0	—
3g		4.0	—
3h		48	1.2
3i		10	—
3j		0.7	—
3k		3.9	—
3l		1.5	—

[a] K_{ass} represents the apparent association constant, as measured by the methods of Smith et al.[5]

[b] 2× PT is defined as the concentration of compound required to double the time for clot formation in the prothrombin time assay.

sults in decreased affinity for fXa as shown in compounds **3k** and **3l**.

A few compounds were evaluated for their anticoagulant potency in human plasma. The compounds with PT data show that as the binding affinity for fXa increases, the concentration of compound required to double the time for clot formation in plasma decreases. Compounds **3c** and **3h** are representative of this trend. In contrast, although **3h** displays an order of magnitude weaker binding affinity for fXa than **1**, a slightly lower concentration is required to double the clot formation time in plasma.

The crystal structure of compound **3h** is shown in Figure 2. The extended P4 domain is shown to bind in the S4 pocket as predicted. A possible explanation for the decrease in activity for **3h** may be that overall the P4 group is positioned in a less favorable arrangement than the equivalent group in compound **1** (Fig. 1).

Specifically, in **3h** the lower piperidine ring is positioned perpendicular with respect to the face of the Trp215 indole sidechain, instead of laying directly on it as in **1**. This will reduce the hydrophobic contact between the ligand and the aromatic residues in this region. Additionally, the potentially charged amino group appears to be partially buried and less solvent exposed in **3h** compared to **1**.

We combined the most potent P4 element in this SAR with a couple of 3-substituted indole P1 constructs. Preparation of compounds **7** and **8** was carried out utilizing the same chemistry as in Scheme 1. The binding affinity, anticoagulant potency, *c*log *P*,[6] and oral exposure data[7] compared to **1** and **3h** are shown in Table 2.

Addition of a methyl group or a chlorine atom in the 3 position of the indole results in increased fXa binding affinity of 2.6-fold and 17-fold, respectively. However, the anticoagulant potencies of **7** and **8** are similar to that of compound **3h**. This reduced anticoagulant activity could be due to the increased lipophilicity of these molecules as shown by the calculated *c*log *P* values. The oral exposure of **3h** is greater than those for compounds **7** and **8**. In addition, the plasma exposure of compound **3h** is greater than that of **1** and has a similar *c*log *P* value.

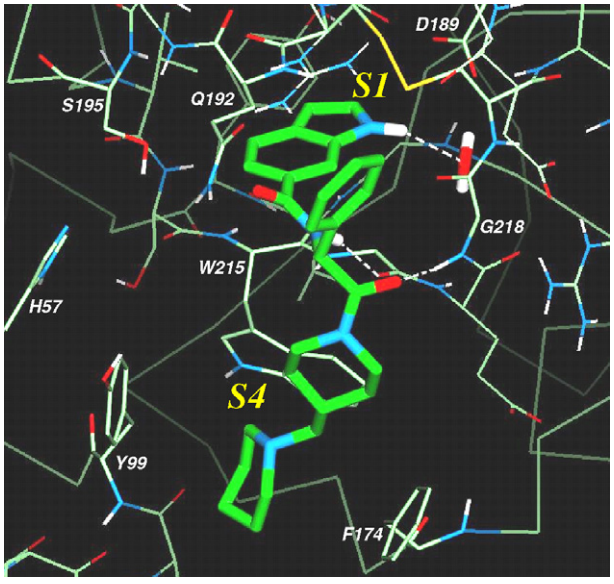
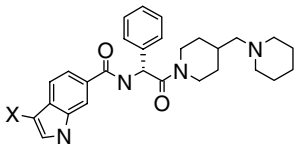


Figure 2. Factor Xa complex with compound **3h**. X-ray crystal structure (2.40 Å resolution).

Table 2. Human factor Xa binding affinity, anticoagulant activity, *c*log *P*, and oral exposure properties of various P1 indole constructs compared to **1**



Compound	X	K_{ass}	*c*log *P*	2× PT (μM)	C_{max} (μM)[a]	AUC (0–7 h) (μg h/ml)[a]
3h	H	48	4.3	1.3	4.7	23
7	Me	110	4.8	1.5	2.4	14
8	Cl	710	5.2	1.1	1.7	10
1	—	450	4.2	1.5	4.0	19

[a] C_{max} and AUC values were pharmacodynamically determined measuring anti-Xa equivalents in rat plasma. Animals were dosed by oral administration at 20 mpk ($n = 3$).

In summary, a series of D-phenylglycine-based inhibitors modifying the terminal P4 element were synthesized and identified as fXa inhibitors. The SAR indicates that extension of the P4 element with or without rings containing hydrogen bonding elements does not increase factor Xa binding affinity relative to **1**. However, the study results in the identification of **3h**, a compound that displays similar anticoagulant activity to **1** while possessing an order of magnitude less binding affinity. Increased affinity for fXa is achieved by incorporating a 3-substituent on the indole P1 domain of this series. The oral exposure of **3h** is found to be greater than those of **7** and **8**, but similar to **1**. Within this series, compounds that display lower exposure tend to be more lipophilic as determined by *c*log *P* values.

Acknowledgments

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References and notes

1. Coleman, R. W.; Marder, V. J.; Salzman, E. W.; Hirsch, J. In *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*; Coleman, R. W., Marder, V. J., Salzman, E. W., Hirsh, J., Eds., 3rd ed.; J.P. Lippincott: Philadelphia, 1994; pp 3–18.
2. (a) Quan, M.; Smallheer, J. *Curr. Opin. Drug Discov. Dev.* **2004**, *7*, 460; (b) Walenga, J. M.; Jeske, W. P. *Curr. Opin. Invest. Drugs* **2003**, *4*, 272; (c) Gould, W. R.; Leadley, R. J. *Curr. Pharm. Design* **2003**, *9*, 2337; (d) Walenga, J. M.;

Jeske, W. P.; Hoppenstedt, D.; Fareed, J. *Meth. Mol. Med.* **2004**, *93*, 95.
3. (a) Jones, S. D.; Liebescheutz, J. W.; Morgan, P. J.; Murray, C. W.; Rimmer, A. D.; Roscoe, J. M. E.; Waszkowycz, B.; Welsh, P. M.; Wylie, W. A.; Young, S. C.; Martin, H.; Mahler, J.; Brady, L.; Wilkinson, K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 733; (b) Sheehan, S. M.; Masters, J. J.; Wiley, M. R.; Young, S. C.; Liebescheutz, J. W.; Jones, S. D.; Murray, C. W.; Franciskovich, J. B.; Engel, D. B.; Weber, W. W.; Marimuthu, J.; Kyle, J. A.; Smallwood, J. K.; Farmen, M. W.; Smith, G. F. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2255.
4. Masters, J. J.; Weber, W. W.; Franciskovich, J. B.; Wiley, M. R.; Halligan, N. R.; Camp, N. P.; Jones, S. D.; Richards, S.; Lyons, A.; Morgan, P. J.; Liebescheutz, J. W.; Murray, C. W.; Young, S. C.; Chirgadze, N. Y.; Briggs, S. L.; Craft, T. J.; Towner, R. D.; Smallwood, J. K.; Smith, G. F. Abstract of Papers, 224th National Meeting of the American Chemical Society, Boston, MA, August 18–22, 2002; American Chemical Society: Washington, DC, 2002; MEDI-284.
5. Smith, G. F.; Shuman, R. T.; Craft, T. J.; Gifford-Moore, D. S.; Kurz, K. D.; Jones, N. D.; Chirgadze, N.; Hermann, R. B.; Coffman, W. J.; Sandusky, G. E.; Roberts, E.; Jackson, C. V. *Semin. Thromb. Hemost.* **1996**, *22*, 173.
6. CLOGP, version 4.72, Daylight Chemical Information Systems Inc., Aliso Viejo, CA 92656.
7. *Pharmacodynamic surrogate of Xa inhibitor oral exposure*. Fisher rats were fasted overnight, followed by a single administration of test compounds suspended in acacia or CMC/Tween 80, by oral gavage. Blood was collected at specified time-points in citrate for generation of plasma at 2000*g* for 15 min at 10 °C. Compounds were weighed out to the nearest 0.001 mg, adjusted for salt form and purity, and dissolved in GC–MS or HPLC grade methanol (OD at 350 nm < .001) with brief sonication in a water bath. Compounds were serially diluted in methanol and aliquoted into pooled plasma from vehicle treated animals in triplicate, for use as calibration standards. A solution for human factor Xa was prepared by dissolving 18.8 mg of fatty acid-free bovine serum albumin (Sigma RIA grade A-7888) in 15 ml of 1× TBS. A solution of Xa substrate, S-2222 (MidWest Bio-Tech, South Bend, IN), was diluted to 0.5 mg/ml in 18.2 MΩ purified water. Water (5 μl) was added to all wells reserved for standards. Methanol (5 μl of 50%) was added to wells reserved for samples of unknown compound level. Ex vivo plasma (25 μl) from animals dosed with compounds was thawed at 4 °C. Samples and calibration standards were mixed, incubated in a 37 °C water bath for 15 min, and diluted into individual wells of a 96-well, polystyrene, uncoated assay plate containing 50 μl of 3× TBS. Substrate solution was then added to all wells, the plate was placed on mixer for 1 min, and a pre-read was collected. Human factor Xa (Enzyme Research Labs HFXa1240, titrated) was thawed at ambient temperature immediately before use and aliquoted into vehicle at 0.4 U/ml. The factor Xa solution was then aliquoted (20 μl) using a multi-channel pipette to start the enzyme reaction. Plates were read on a Spectromax 250 plate reader (Molecular Devices, Sunnyvale, CA) at 405 nm kinetically for 10 min at 11 s/cycle, immediately followed by an end-point read. Slopes of OD/min were plotted against the known concentrations of calibration standards and samples of unknown concentration were interpolated from four parameter logistic fits of standard curves. Compound concentrations of ex vivo samples from dosed animals were used in SigmaPlot for the calculation of AUCs using a SigmaPlot rev 8.1 area algorithm.