Bioorganic & Medicinal Chemistry Letters 20 (2010) 1373–1377

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Phenyltriazolinones as potent factor Xa inhibitors

Mimi L. Quan *, Donald J. P. Pinto, Karen A. Rossi, Steven Sheriff, Richard S. Alexander, Eugene Amparo, Kevin Kish, Robert M. Knabb, Joseph M. Luettgen, Paul Morin, Angela Smallwood, Francis J. Woerner, Ruth R. Wexler

Research and Development, Bristol-Myers Squibb, PO Box 5400, Princeton, NJ 08543-5400, United States

ARTICLE INFO

Article history:
Received 17 November 2009
Revised 27 December 2009
Accepted 4 January 2010
Available online 11 January 2010

Keywords:
Factor Xa inhibitors
Coagulation

ABSTRACT

We have discovered that phenyltriazolinone is a novel and potent P_1 moiety for coagulation factor Xa. X-ray structures of the inhibitors with a phenyltriazolinone in the P_1 position revealed that the side chain of Asp189 has reoriented resulting in a novel S_1 binding pocket which is larger in size to accommodate the phenyltriazolinone P_1 substrate.

Cardiovascular diseases are the leading cause of death in developed countries, and most cardiovascular events are primarily due to thrombosis.[1] Despite the extensive efforts to discover and develop new antithrombotic agents,[2] warfarin remains the only approved oral anticoagulant in the United States.[3] Other agents such as heparin and fondaparinux are available only by parenteral administration. Limitations of these agents have prompted extensive research for novel anticoagulants.[3] Factor Xa (fXa) has been a major focus of pharmaceutical intervention in the past decade because of its central and unique position in the coagulation cascade.[4] Significant progress has been made in the discovery and development of fXa inhibitors as anticoagulants. Several small molecule fXa inhibitors have been in phase III clinical trials. Among them the first oral fXa inhibitor, rivaroxaban, has recently been approved in both Europe and Canada for the prophylaxis of venous thromboembolism.[5]

Our efforts to discover and develop orally active inhibitors of fXa led to clinical candidates DPC423 (**1**),[6] razaxaban (**2**),[7] and apixaban (**3**)[8] (Fig. 1). Razaxaban and apixaban were studied in phase II clinical trials and shown to be efficacious in the treatment of deep vein thrombosis.[9] Apixaban, which has an overall better pharmacokinetic profile, is currently in phase III clinical trials for both venous and arterial indications.[9b] The discovery effort which ultimately led to apixaban significantly advanced our understanding of moieties that were sufficiently tolerated on the P_1 phenyl group.[10,11] As part of our efforts to further diversify the P_1 portfolio, we discovered a novel phenyltriazolinone P_1 moiety as shown in structures **4b** and **5**. Herein we describe the synthesis and X-ray crystal structures of these potent and selective fXa inhibitors containing a phenyltriazolinone in the P_1 position.

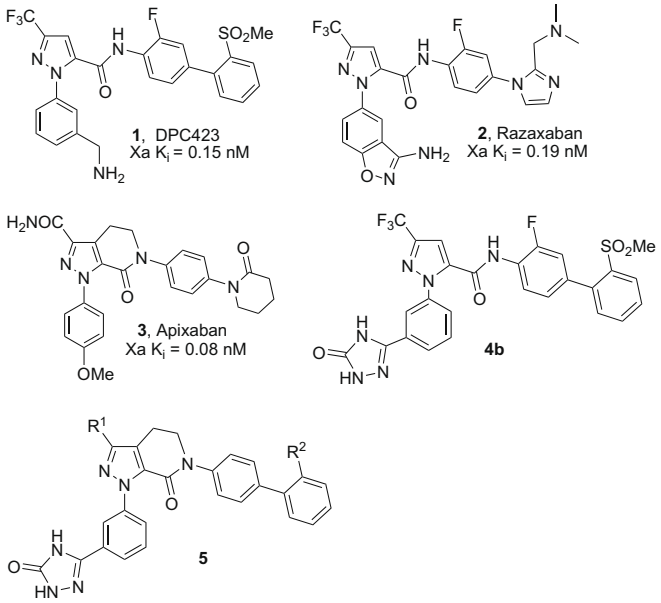

Figure 1. Structures of key compounds.

* Corresponding author. Tel.: +1 609 818 5301; fax: +1 609 818 3331.
E-mail address: mimi.quan@bms.com (M.L. Quan).

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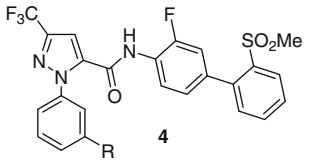
doi:10.1016/j.bmcl.2010.01.011

Table 1
P_1 SAR in DPC423 series



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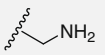
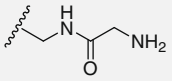
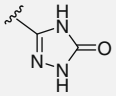
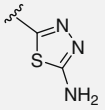
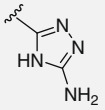
Compd	R	fXa K_i (nM)	IIa K_i (nM)	Trpsin K_i (nM)
DPC423		0.15	6000	60
4a		9	>21,000	1200
4b		0.5	>21,000	>2500
4c		46	>21,000	>2500
4d		15	4900	160

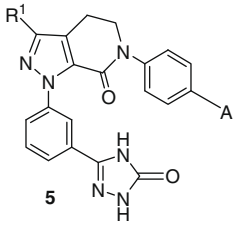
The K_i's obtained from purified human enzymes and are averaged from multiple determinations ($n = 2$).

The syntheses of compounds listed in Tables 1 and 2 are shown in Schemes 1–3. The glycinamide **4a** was prepared from DPC423[6a] in a two step manner. DPC423 was coupled to NBocGlycine and deprotected with TFA to afford compound **4a**. Compounds **4b–d** were prepared from compound **7**[6a] (Scheme 1). The cyano group in **7** was readily converted to the triazolinone derivative **4b** by treatment with gaseous HCl in methanol, followed by treatment with semicarbazide/*N*-methylmorpholine in dioxane at reflux. Using a similar but modified protocol, the aminothiadiazole **4c** and aminotriazole **4d** were also prepared as shown in Scheme 1.

Triazolinones **5a–f** were prepared in a similar fashion as shown in Scheme 2. Compound **8** was synthesized according to the procedures described in Ref. 10. The cyano group was converted to triazolinone **9** as described above. Suzuki coupling of **9** with 2-formylphenylboronic acid (**10**), followed by reductive amination with appropriate amines afforded compounds **5c–f** in Table 2. Suzuki coupling of **9** with 2-methylsulfonylphenylboronic acid (**11**) provided compound **5a**. Ullmann coupling of compound **8** with imidazole **12** gave compound **13**, which was converted to triazolinone **5b** via methods described above.

Scheme 3 shows the synthesis for compound **5g** wherein the CF_3 on the pyrazole was replaced with a carboxamide moiety. Compounds **14** and **15** were prepared according to the procedures described in Ref. 10. Reaction of **14** and **15** with triethylamine in refluxing toluene gave bicycle **16**. The ester in compound **16** was converted to the carboxamide to yield **17**. Formation of the triazolinone followed by Suzuki reaction and reductive amination as described previously produced compound **5g** in Table 2.

Table 2
SAR of triazolinone P_1 in the bicyclic series



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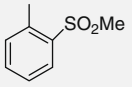
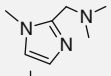
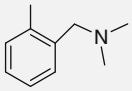
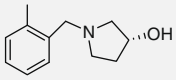
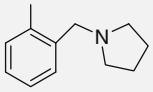
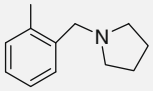
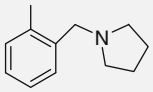
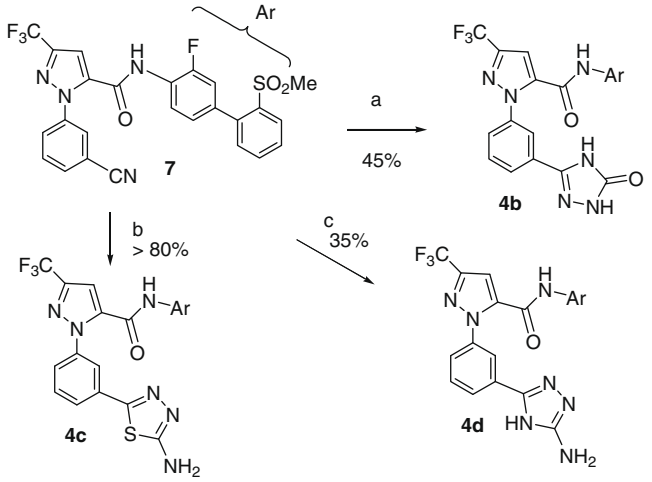
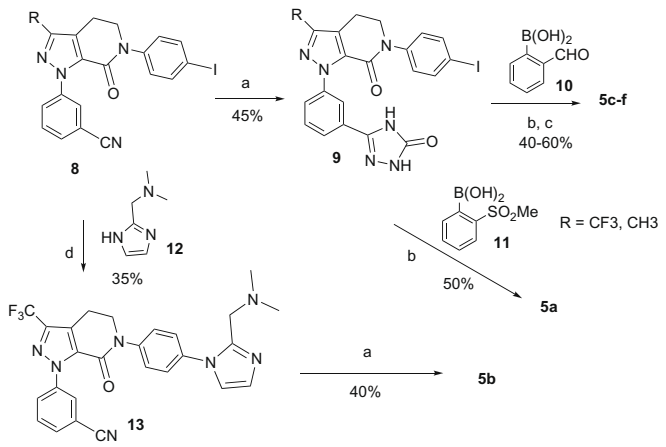
Compd	R^1	A	fXa K_i (nM)	IIa K_i (nM)	PT EC2x (μM)
5a	CF_3		2.2	>6000	>80
5b	CF_3		2.0	>6000	11
5c	CF_3		1.1	>6000	33
5d	CF_3		0.84	>6000	59
5e	CF_3		0.40	>6000	39
5f	CH_3		8.1	>6300	Not tested
5g	**$CONH_2$**		**0.25**	**>6000**	**4.4**
Razaxaban			**0.19**	**540**	**2.1**
Apixaban			**0.08**	**3100**	**3.8**

The K_i's obtained from purified human enzymes and are averaged from multiple determinations ($n = 2$). All compounds have $K_i > 4200$ nM for trypsin.



Scheme 1. Reagents and conditions: (a) (1) HCl/MeOH; (2) NMM, semicarbazide, dioxane, reflux; (b) thiosemicarbazide, TFA, reflux; (c) (1) HCl/MeOH; (2) cyanoguanidine, K_2CO_3, AcOH, dioxane, 140 °C.

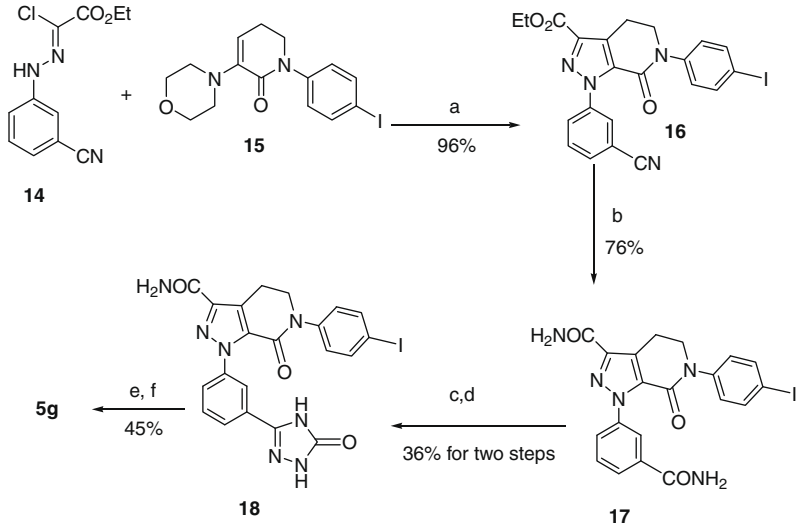


Scheme 2. Reagents and conditions: (a) (1) HCl/MeOH; (2) NMM, semicarbazide, dioxane, reflux; (b) (1) $Pd(PPh_3)_4$, 2 M Na_2CO_3, toluene/EtOH or DME/H_2O, reflux; (c) amine, $NaCNBH_3$, MeOH; (d) CuI/K_2CO_3, 1,10-phenantholine, DMSO, 130 °C, 12 h.

The suboptimal trypsin selectivity profile of DPC423 prompted us to investigate additional P_1 moieties for potency and selectivity. Glycinamide P_1 (**4a**) showed low nanomolar binding affinity against fXa which suggested that this P_1 moiety sits deeper into the S_1 pocket of fXa. To further elaborate on this finding, a set of constrained heterocyclic analogs were evaluated (**4b–d**). Although most of these compounds were less potent, the triazolinone compound **4b** retained the fXa affinity with a fXa K_i of 0.5 nM (Table 1). With the exception of **4d**, these compounds show significantly weaker affinity for thrombin and trypsin compared with DPC423.

We next evaluated the triazolinone P_1 group appended to the bicyclic pyrazole scaffold of apixaban. As shown in Table 2, compounds **5a–e** with a trifluoromethyl substitution on the bicyclic pyrazole core achieved low nanomolar to sub-nanomolar affinity. However, weak in vitro anticoagulant activity was observed with these compounds. This was attributed to higher lipophilicity which contributes to higher protein binding (for a discussion of the effect of plasma protein binding on translation from fXa K_i to in vitro anticoagulant activity see Ref. 7a). To lower the protein binding of compound **5e**, the C-3 pyrazole trifluoromethyl group was replaced with C-3 methyl and C-3 carboxamide moieties, which have previously been shown to impact higher free fraction in related compounds.[8a] While the methyl analog **5f** lost fXa affinity, the carboxamide compound **5g** had a fXa K_i of 0.25 nM and improved in vitro anticoagulant potency (PT EC2x = 4.4 μM).[12] In comparison with razaxaban (fXa K_i = 0.19 nM and PT EC2x = 2.1 μM), compound **5g** was within twofold in terms of in vitro anticoagulant activity, and despite a threefold difference in fXa affinity, was comparable in the PT assay to apixaban (fXa K_i = 0.08 nM and PT EC2x = 3.8 μM).

Modeling of the phenyltriazolinone into the S_1 pocket of the fXa active site indicated that the phenyltriazolinone moiety was not expected to fit in the S_1 pocket. The increased size of the triazolinone moiety was predicted to result in steric clashes with Asp189. To better understand the observed results, X-ray structures of compounds **4b, 5a,** and **5d–f** bound to human fXa were determined.[13] All of these compounds bind to fXa in a similar mode as depicted in Figure 2, which shows the overlay of all five compounds in the fXa active site. The X-ray structures show all compounds maintain the general binding mode previously observed with other pyrazole-based fXa inhibitors except in the



Scheme 3. Reagents and conditions: (a) TEA, toluene, reflux; (b) formamide, NaOMe, DMF, 100 °C; (c) HCl/MeOH; (d) NMM, semicarbazide, dioxane, reflux; (e) 2-formylphenylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, toluene/EtOH or DME/H_2O, reflux; (f) amine, $NaCNBH_3$, MeOH.

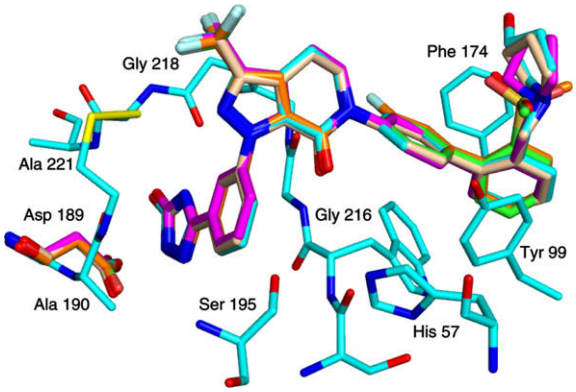


Figure 2. Overlay of the crystal structures of **4b** (orange), **5a** (green), **5d** (cyan), **5e** (magenta), and **5f** (tan) in the fXa active site (Figure created with PyMol[14]).

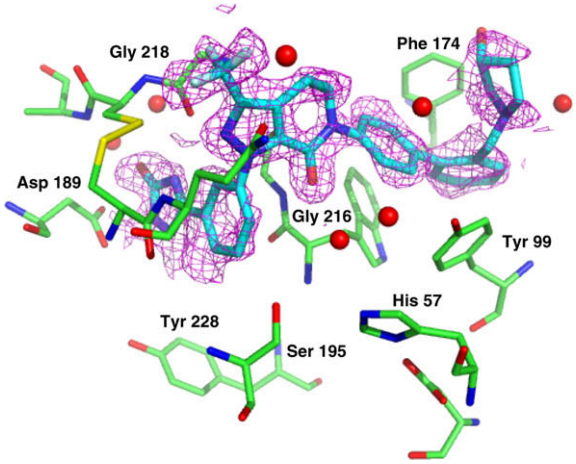


Figure 3. Crystal structure of **5d** bound to human fXa: Initial 2Fo–Fc electron density (magenta) contoured at 1σ is shown with the final model of compound **5d**. Carbon atoms are shown in cyan for compound **5d** and green for fXa. Selected water molecules are shown as spheres. Figure created with PyMol.[14]

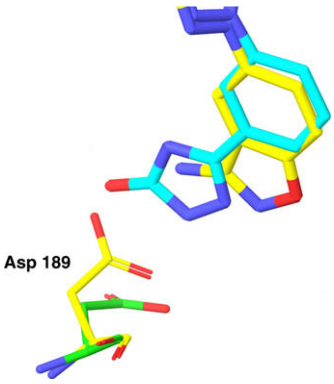


Figure 4. Overlay of the phenyltriazolinone (Cyan) and the aminobenzisoxazole (yellow) moieties in the S1 site.

S_1 pocket. With all five compounds, the Asp189 side chain in the S_1 binding site has rotated away from its normal location. This movement creates a novel larger pocket to accommodate the phenyltriazolinone. Figure 3 shows the crystal structure of compound **5d** with the initial electron density of the ligand. An overlay of the phenyltriazolinone with the aminobenzisoxazole[7a] clearly

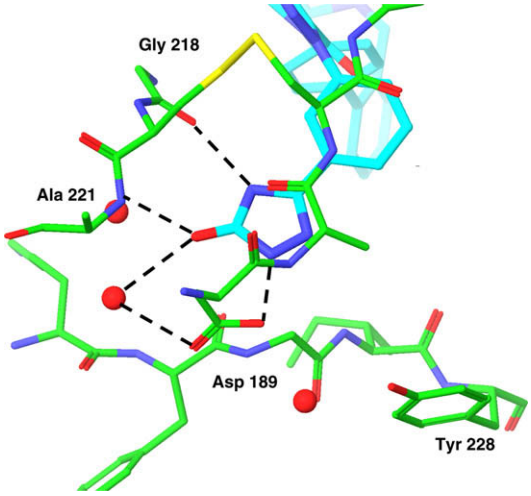


Figure 5. Interactions of **18** in the S_1 pocket.

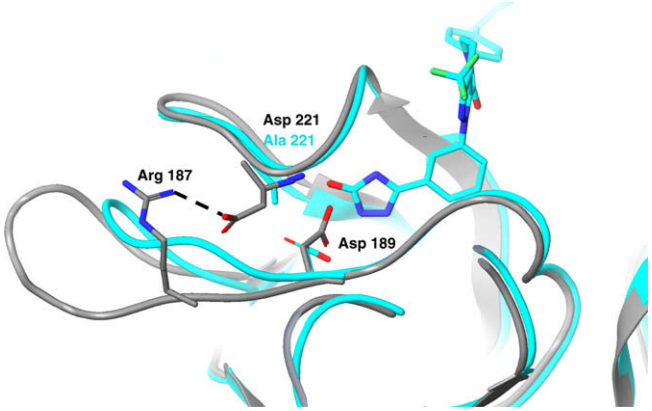


Figure 6. Comparison of fXa (cyan) to thrombin (grey).

shows that Asp189 has moved and the triazolinone has extended much deeper into the S_1 site (Fig. 4).

Figure 5 shows the detailed interactions of the phenyltriazolinone in the S_1 pocket. One of the nitrogen atoms of the triazolinone forms a H-bond with the Gly218 carbonyl. The triazolinone carbonyl forms two H-bonds, one directly with Ala221 nitrogen and another with one of the carboxylic oxygens of Asp189 side chain through a water molecule. Another nitrogen of the triazolinone ring forms a H-bond with the second oxygen of Asp189 side chain which in turn forms a H-bond with Tyr223 OH through an additional water molecule. This network of H-bonds enables the triazolinone moiety to have strong interactions with the enzyme.

The selectivity of the triazolinones against trypsin can be rationalized by a change in residue in the S_1 pocket. FXa has Ala190 adjacent to Asp189, whereas trypsin has a bulkier serine residue in this position. These larger S_1 substituents are unable to bind when the serine is present, therefore yielding selectivity over trypsin. Comparison of the S_1 pockets of fXa and thrombin reveals a possible reason for the selectivity of the triazolinones against thrombin. The fXa structure shows the movement of Asp189; however, thrombin would be unable to adopt this conformation due to differences in residues near Asp189 that would create unfavorable steric and electronic interactions with Asp189. The triazolinone P_1 moiety moves Asp189 deeper into the fXa S_1 pocket, and puts it adjacent to Ala221. In thrombin, this residue is Asp221, which partially occludes the pocket and provides an unfavorable electrostatic environment for Asp189 to rotate towards, resulting in improved selectivity against thrombin (Fig. 6).

In summary, we have discovered the first examples of movement of Asp189 upon binding to fXa inhibitors. A series of novel and potent fXa inhibitors with a phenyltriazolinone P_1 moiety were identified. Although the general binding mode compared to our previously-reported fXa inhibitors was maintained, a larger cavity to accommodate this P_1 moiety was created by the movement of the Asp189 side chain. X-ray structures revealed an intricate network of H-bonds between the triazolinone and the enzyme including several structural water molecules.

Acknowledgements

The authors thank Tracy Bozarth for clotting times, and Joanne Smallheer for helpful discussions and suggestions.

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