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Design, synthesis, and thrombin-inhibitory activity of pyridin-2-ones as P_2/P_3 core motifs

Stephen Hanessian,[a,*] Daniel Simard,[a] Malken Bayrakdarian,[a] Eric Therrien,[a] Ingemar Nilsson[b,*] and Ola Fjellström[b]

[a]*Department of Chemistry, Université de Montréal, C.P. 6128, Succ. Centre-Ville, Montréal, Que., Canada H3C 3J7*
[b]*AstraZeneca R&D Mölndal, Medicinal Chemistry Mölndal, Sweden*

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Abstract—Guided by available X-ray crystal structure data on the serine protease thrombin, a series of pyridin-2-one derivatives were designed and synthesized having diverse functionality at the P_1 and P_3 sites. Potent in vitro activity against thrombin, with excellent selectivity over trypsin was found for selected analogues.

Thromboembolic disorders are the major cause of mortality and morbidity in the industrial world.[1] Closely linked to the clinical syndromes of thromboembolism is an excessive stimulation of the coagulation cascade. The serine protease thrombin plays a critical role in the blood coagulation cascade. Predominantly, it is the key terminal protease and holds a central role in both hemostasis and thrombosis.[1] Thrombin effectively catalyzes the proteolytic cleavage of the soluble plasma-protein fibrinogen to form insoluble fibrin leading to clot formation. Considerable efforts have therefore been devoted to the discovery of safe, orally active inhibitors of this enzyme in order to replace those that are used nowadays.[1]

X-ray crystal structures of thrombin-inhibitor complexes have offered attractive options for the design and synthesis of structure-based potential inhibitors.[2] Indeed, several peptidomimetic-type drug candidates known to interact with thrombin at specific sites are in advanced stages of clinical studies.[3] More recent efforts have concentrated on non-peptidic inhibitors based on heteroaromatic or heterocyclic core structures.[4]

As part of our ongoing project in the discovery of new potential thrombin inhibitors, we have previously reported on the design, synthesis, and X-ray cocrystal structure of indolizidinones with thrombin.[5] Low nanomolar inhibition of thrombin validated the initial design concept of an indolizidinone motif with strategically placed substituents as a constrained mimic of the natural substrate D-Phe-Pro-Arg.[6] We have also reported on related indolizidinones designed to target Factor VIIa.[7] Simpler indolizidinones and related sultams[8] were also synthesized as potential thrombin inhibitors. The marine natural products Dysinosin A,[9] Oscillarin,[10] and Chlorodysinosin A[11] were recently synthesized in our laboratories to evaluate their inhibitory activity against thrombin and Factor VIIa. Truncated analogues of these natural products containing a 2-carboxy-octahydroindole P_2 core unit were also recently reported.[12]

In a previous publication, we reported on the design, synthesis, and X-ray cocrystal structure of low nanomolar phenolic thrombin inhibitors.[13] We now report on the design, synthesis and thrombin-inhibitory activity of a series of amines and sulfonamides appended to a trisubstituted pyridin-2-one core structure represented by the generic expression **1** (Fig. 1). The requisite P_1, P_2, and P_3 interactions with their respective subsites in the enzyme as suggested by molecular modeling augured well for anticipating in vitro activity as well.

The pyridin-2-one core motif incorporating diverse functionality in the P_1/P_3 positions was synthesized as shown in Scheme 1. Thus, the 2-methoxy-4-methyl-pyridine-3-carbaldehyde[14] **2** was converted into the acetonitrile analogue **3** in 57% overall yield using a three-step

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* Corresponding authors. Tel.: +1 514 343 6738; fax: +1 514 343 5728 (S.H.); tel.: +46 31 7761161; fax: +46 31 7763839 (I.N.); e-mail addresses: stephen.hanessian@umontreal.ca; ingemar.nilsson@astrazeneca.com

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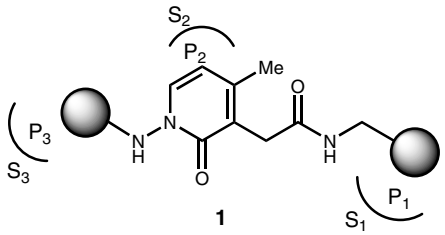


Figure 1. Prototype of pyridin-2-one core with variations in the P_1 and P_3 subunits.

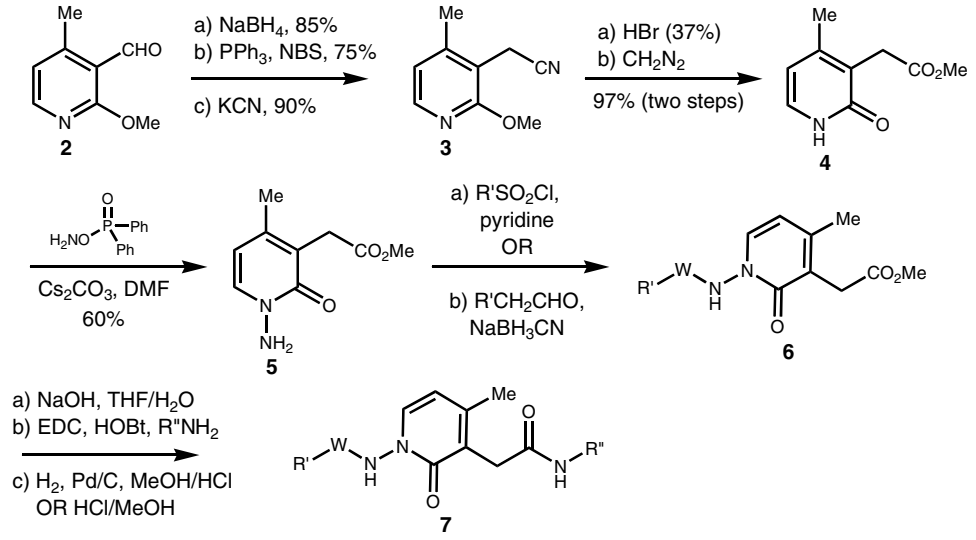
procedure. Acidic hydrolysis of **3** furnished the expected pyridone which was esterified with diazomethane to give the methyl ester **4** in excellent overall yield. Then, the *N*-amino functionality was introduced using a phosphine-based amino transfer reagent[15] yielding the corresponding *N*-amino pyridone **5**. Arylsulfonylation or alkylation via reductive amination of **5** with the desired P_3 appendage afforded **6**. Hydrolysis of the ester followed by amide formation, and subsequent deprotection yielded the analogue **7**. In order to provide sufficient diversity, analogues featuring different aromatic P_1 substituents were prepared using the general protocol (Fig. 2).

The sets of sulfonamides and amines harboring the pyridin-2-one core motif exhibited excellent purities by LC/MS. The IC_{50} values of the synthesized analogues in the 4-amidinobenzyl P_1 series (**A**) are listed in Table 1.

The pyridin-2-one analogues in this series exhibited IC_{50} values in the range of 0.02–2.5 μM with various selectivity over trypsin (Table 1). The amines **8**, **9**, and **10** (Table 1, entries 1–3) showed excellent antithrombin activity with IC_{50} below 100 nM. Thus, the best compound in this series is the *o*-methyl phenethyl amine **8** with an IC_{50} of 23 nM against thrombin. Several substituted sulfonamides exhibited IC_{50} values in the range of 0.3–1.2 μM (Table 1, entries 4–9).

Unfortunately, no clear-cut SAR could be observed by varying the position of aromatic substituents. The addition or deletion of a methyl group in the aromatic ring led to a fourfold decrease of the in vitro activity (Table 1, compare entries 1, 2, and 3). In addition, the nature of the P_3 amine seems important for binding as observed by a 20-fold potency increase when the sulfonamide functionality is replaced by an alkylamine (Table 1, compare entries 2 and 11). Although the activities could not be directly correlated with the nature or position of aromatic substitution on the P_3 part, we were intrigued that analogue **8** showed good antithrombin activity with a 42-fold selectivity over trypsin.

Table 2 lists the IC_{50} values for the pyridin-2-one analogues in the series **B**. Equipotent antithrombin activity was observed for the substituted phenethyl amines (Table 2, entries 1–2). However, aromatic or heterocyclic sulfonamides seemed detrimental to thrombin inhibition (Table 2, entries 3–4). The same trend was observed with the 2-amino-4-aminomethyl pyridine P_1 subunit (series **C**, Table 3), as well as with the 2-amino-methyl-3-fluoro-6-methyl pyridine P_1 subunit (series **D**, Table 4), although the phenethyl amine **28** did not show any activity against thrombin in the series **D**.[16]



Scheme 1. General protocol for the synthesis of the pyridin-2-one analogues.

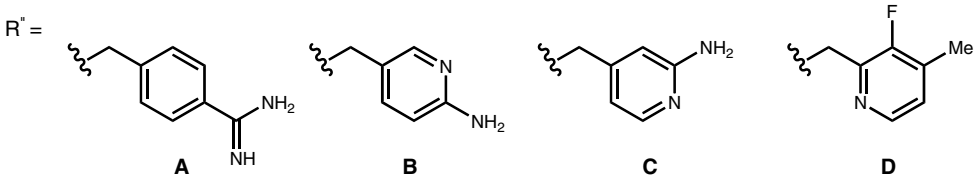
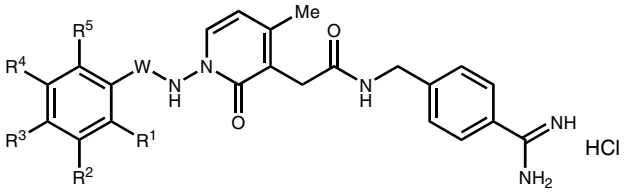


Figure 2. Different P_1 substituents.

Table 1. IC_{50} values for the 4-amidinobenzyl P_1 subunit (series **A**)

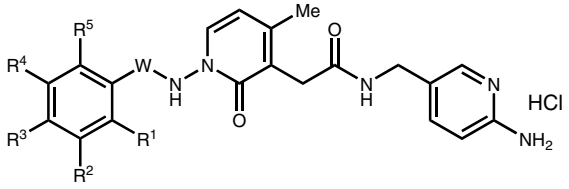


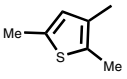
IC_{50} (μM)

Entry	Compound	W	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)		Ratio[a]
								Thrombin	Trypsin	
1	**8**	CH_2CH_2	Me	H	H	H	H	0.023	0.963	42
2	**9**	CH_2CH_2	H	H	H	H	H	0.091	1.420	16
3	**10**	CH_2CH_2	Me	H	H	Me	H	0.092	2.140	23
4	**11**	SO_2	OMe	H	Cl	H	H	0.307	4.130	13
5	**12**	SO_2	Me	H	H	Me	H	0.578	7.130	12
6	**13**	SO_2	H	Cl	Cl	H	H	0.885	10.300	12
7	**14**	SO_2	H	OMe	H	H	H	1.054	15.600	15
8	**15**	SO_2	OMe	H	Me	H	H	1.180	2.020	1.7
9	**16**	SO_2	CH=CH–CH=CH		H	H	H	1.243	17.100	14
10	**17**	SO_2	H	H	OMe	H	H	1.846	3.410	1.8
11	**18**	SO_2CH_2	H	H	H	H	H	1.919	15.200	7.9
12	**19**	SO_2	H	H	H	H	H	2.471	5.770	2.3

[a] Ratio of (IC_{50} trypsin)/(IC_{50} thrombin).

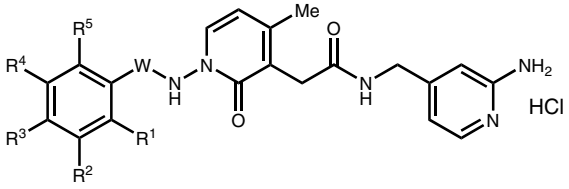
Table 2. IC_{50} values for the 2-amino-5-aminomethyl pyridine P_1 subunit (series **B**)



Entry	Compound	W	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)		Ratio[a]
								Thrombin	Trypsin	
1	**20**	CH_2CH	H	CF_3	H	H	H	2.706	> 44.400	>16
2	**21**	CH_2CH	Me	H	H	F	H	3.091	> 44.400	>14
3	**22**	SO_2	OMe	H	OMe	H	H	>44.400	> 4.400	—
4	**23**	SO_2						>44.400	>44.400	—

[a] Ratio of (IC_{50} trypsin)/(IC_{50} thrombin).

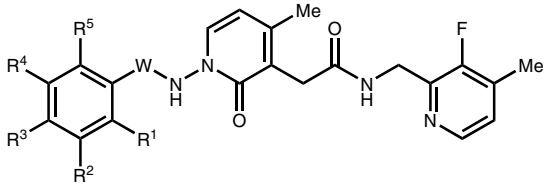
Table 3. IC_{50} values for the 2-amino-4-aminomethyl pyridine P_1 subunit (series **C**)



Entry	Compound	W	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)		Ratio[a]
								Thrombin	Trypsin	
1	**24**	SO_2CH_2	H	H	Cl	H	H	>44.400	>44.400	—
2	**25**	SO_2	H	H	Et	H	H	> 44.400	>44.400	—

[a] Ratio of (IC_{50} trypsin)/(IC_{50} thrombin).

Table 4. IC_{50} values for the 2-aminomethyl-3-fluoro-4-methyl pyridine P_1 subunit (series **D**)



Entry	Compound	W	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)		Ratio[a]
								Thrombin	Trypsin	
1	**26**	SO_2	Me	H	H	H	Cl	>44.400	>44.400	—
2	**27**	SO_2	SO_2Me	H	H	H	H	>44.400	>44.400	—
3	**28**	CH_2CH_2	F	H	H	Cl	H	>44.400	>44.400	—
4	**29**	SO_2						>44.400	>44.400	—

[a] Ratio of (IC_{50} trypsin)/(IC_{50} thrombin).

In conclusion, we have shown that an *N*-amino pyridin-2-one can be used as a P_2/P_3 core motif for thrombin inhibition. In general, the phenethyl amines obtained by a reductive amination protocol were more active against thrombin compared to the corresponding sulfonamides. In addition, the 4-amidinobenzyl substituent was found to be the most effective amide P_1 subunit (series **A**). We also showed that the selectivity over trypsin can be modulated according to the nature of the P_3 subunit. The simplicity of these structures and their relative ease of synthesis should pave the way to a better understanding of the relative roles of the P_2/P_3 subunits, and the subtle influences of aromatic substitutions on the enzymatic activity.

Acknowledgments

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