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Heterocyclic Thrombin Inhibitors. Part 1: Design and Synthesis of Amidino-Phenoxy Quinoline Derivatives

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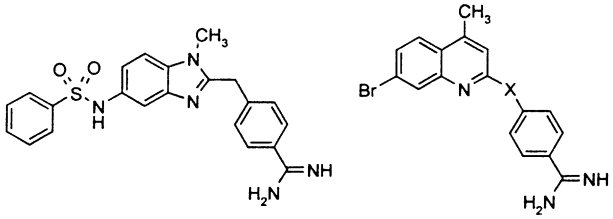
Abstract—Amidino-phenoxy quinoline derivatives represent a new class of potent thrombin inhibitors with good selectivity and remarkably low molecular weight (M_W: 335–391). X-ray analyses of thrombin-bound inhibitors revealed that enzyme inhibition is mainly based on hydrophobic interactions.

The search for potent and orally active thrombin inhibitors for antithrombotic therapy has been one of the main challenges in medicinal chemistry for more than a decade. In the past synthetic efforts have been concentrated on tripeptide analogues (transition state analogues) and arginine amide derivatives.[1] The early inhibitor types possessed reactive functional groups such as aldehydes, activated ketones or boronic acids. These common structural features may be responsible for several problems like lack of selectivity, side effects, rapid clearance or low oral bioavailability. In recent years new types of non-peptidic thrombin inhibitors have been described having heterocyclic core structures,[2] which are expected to show advantages concerning the required properties in vitro and in vivo.

Recently, we described the design and synthesis of novel benzimidazole derivatives as potent, direct thrombin inhibitors with favorable pharmacokinetics and oral activity.[3] Based on the lead structure **1** we designed a number of alternative heterocyclic analogues, from which the key compound, quinoline derivative **2a**, was discovered (Scheme 1). Surprisingly **2a** showed a 22-fold higher affinity for thrombin compared to the benzimidazole lead **1**, although it lacks the important interaction of the phenyl sulfonamide moiety with the S4-subsite of the enzyme. Herein we wish to report our efforts to optimize the in vitro and in vivo potency, and we present crystal structures of two of these new inhibitors bound to thrombin.

The short and efficient syntheses of amidinophenoxy quinoline derivatives are outlined in Scheme 2. 2-Chloro-4-methyl-7-bromo quinoline[4] was reacted with 4-hydroxy-benzonitrile at 160 °C to yield the cyanophenoxy analogue **6**. This central intermediate was used for Suzuki-type couplings[5] yielding substituted 7-phenyl-quinoline derivatives like **3a**. Similarly, **6** was reacted with (E)-ethyl crotonate under Heck-conditions[6] to afford the cinnamic acid derivatives (e.g., **4a**). Cyclopentyl-substituted quinolines like **5a** were prepared by the following sequence: Palladium-catalyzed coupling of **6** with tributyl allyltin gave the corresponding allyl derivative, which was oxidized with $RuCl_3/NaIO_4$. The resulting phenylacetic acid derivative was converted into the corresponding ethyl ester and dialkylated. The cyano groups in all intermediates were reacted under



Scheme 1. Benzimidazole and quinoline lead structures.

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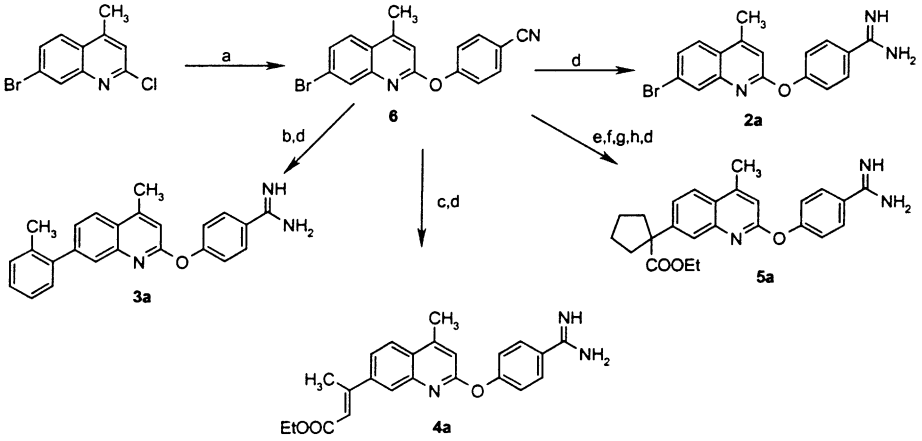


Scheme 2. (a) 4-Hydroxybenzonitrile, DMF, 160 °C, 1 h, 74%. (b) 2-methyl-phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, 110 °C, 6 h, 91%. (c) (E)-ethylcrotonate, $Pd(OAc)_2$, tris-(*o*-tolyl)phosphine, TEA, xylene, 32 h, 150 °C, 23%. (d) (1) HCl (g), EtOH, 25 °C; (2) $(NH_4)_2CO_3$, EtOH, 12 h, 25 °C, 60–95%. (e) allyltributyltin, $Pd(PPh_3)_4$, toluene, 15 h, 120 °C, 96%. (f) $RuCl_3$, $NaIO_4$, $H_2O/CH_2Cl_2/CH_3CN$, 24 h, 25 °C, 40%. (g) carbonyl-diimidazole, THF, EtOH, 2 h, 25 °C, 39%. (h) $Br(CH_2)_4Br$, NaH, DMSO, 1 h, 40 °C, 39%.

Pinner conditions[7] yielding the corresponding amidines. Compounds **2b** and **2c** were prepared on identical routes using 4-amino-benzonitrile or 4-mercapto-benzonitrile in the first step of the reaction sequence.

The starting point for the optimization of in vitro potency was the lead compound **2a** (Scheme 1). Molecular modeling studies of **2a** indicated that the amidino group interacts with the specificity pocket (S1 subsite) via a salt bridge with Asp 189 and the 4-methyl group points into the proximal hydrophobic pocket (S2). As expected, the oxygen atom between the quinoline and benzamidine moieties could be replaced by nitrogen or sulfur with little effect on inhibitory potency (**2b**/**2c** vs **2a**). In the proposed binding mode the inhibitor cannot reach the distal pocket (S4 subsite) due to its small size. In contrast to these compounds nearly all thrombin inhibitors of comparable potency reported in the past contain an additional lipophilic group which contributes to the binding energy via an interaction with the S4 subsite.[8] However, a first report describing nanomolar thrombin inhibitors lacking a group to fill the S4 pocket has been published recently.[9] In order to improve enzyme inhibition we designed substituents for the quinoline position 7, which should place a lipophilic residue into the distal pocket. In addition, these substituents should also contain a polar group interacting with the water phase in order to allow modifications of the inhibitor polarity, which has a strong influence on important parameters like solubility, protein binding and free inhibitor concentration in plasma.[10]

For the first step two different tolyl substituents were introduced at the quinoline position 7 resulting in 11- and 4-fold improvement of enzyme inhibition, respectively, (**3a**/**3b**, Table 1). The X-ray structure of the thrombin/**3c** complex, the amino analogue of **3a** (Fig. 1), confirmed the anticipated binding mode of the proximal half of the inhibitor.[11] Apart from the salt bridge between the amidino group and Asp 189 no polar interaction between the inhibitor and the enzyme is observed. Therefore the high binding affinity is probably mainly based on lipophilic interactions and displacement of water from the active site. The *o*-tolyl ring is turned 72° relative to the quinoline plane and the *o*-methyl group points into the distal pocket. Thereby, a favorable binding geometry is adopted. The *o*-methyl moiety is able to only partially occupy the S4-subsite.

Surprisingly, the *p*-tolyl analogue **3b** also turned out to be a highly potent thrombin inhibitor, although it should not be able to interact with the S4 subsite. Modeling studies based on the **3c**-*co*-crystal structure suggests that the *p*-tolyl group in **3b** should not occupy the S4 subsite but should interact with a lipophilic groove in front of the distal pocket consisting of the aliphatic portions of the side chains of Ile 124 and Glu 217. Obviously the interaction of **3b** with this binding area[8b] makes an important contribution to the overall binding energy.

These findings encouraged us to design additional quinoline derivatives containing small substituents able to interact only with this lipophilic groove. Introduction of an ethyl 2-methylcrotonate residue led to an equally potent inhibitor (**4a**). In contrast, the corresponding

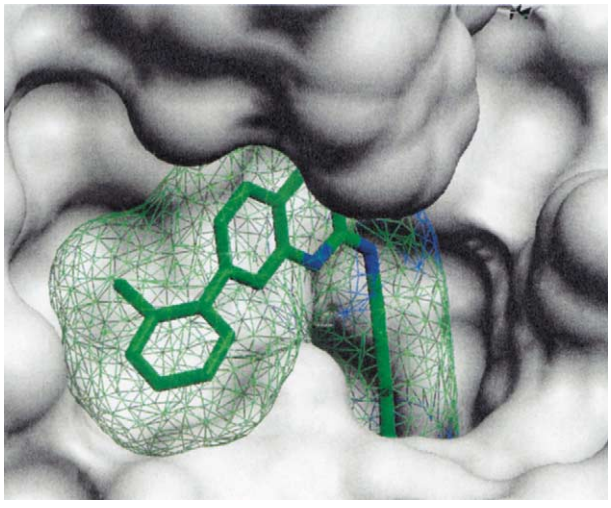
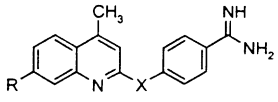
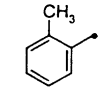
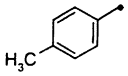
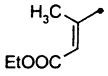
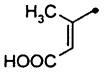
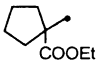
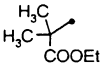
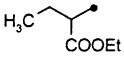


Figure 1. X-ray structure of the thrombin/3c complex.

Table 1. Thrombin inhibition of quinoline derivative



Compd	R	X	Thrombin inhibition IC_{50}, μM	Trypsin inhibition[a] IC_{50}, μM	Plasmin inhibition[a] IC_{50}, μM
3a		O	0.006	2.9	8.3
3b		O	0.018	4.2	12.2
3c		NH	0.037	3.5	14.8
4a		O	0.016	6.7	6.4
4b		O	0.6	8.1	25
5a		O	0.003	0.56	5.8
5b		O	0.006	2.4	20
5c		O	0.01	n.d.	n.d.
5d		O	0.092	n.d.	n.d.
5e		O	3.8	n.d.	n.d.

[a]n.d. = not determined.

acid **4b** was only weakly active indicating that the negatively charged carboxylate disturbs the enzyme-inhibitor interaction.

Several ethyl phenylacetate derivatives (**5a**–**5d**) also turned out to be potent thrombin inhibitors, depending on the substitution at the α-carbon atom. The dialkylated derivatives **5a** and **5b** showed enzyme inhibition in the lower nanomolar range, whereas the mono ethyl derivative **5c** was slightly less active. A comparison with the unsubstituted ethyl phenylacetate **5d** indicates that the alkyl groups in **5a**–**5c** strongly contribute to the enzyme inhibition. As in the case of **4a**/**4b**, transformation of the ester **5d** into the corresponding acid **5e** led to a substantial loss of activity. Most of the quinoline derivatives listed in Table 1 showed good selectivity versus trypsin and plasmin.

In order to gain insight into the molecular interactions of **5a** with thrombin, we co-crystallized this compound

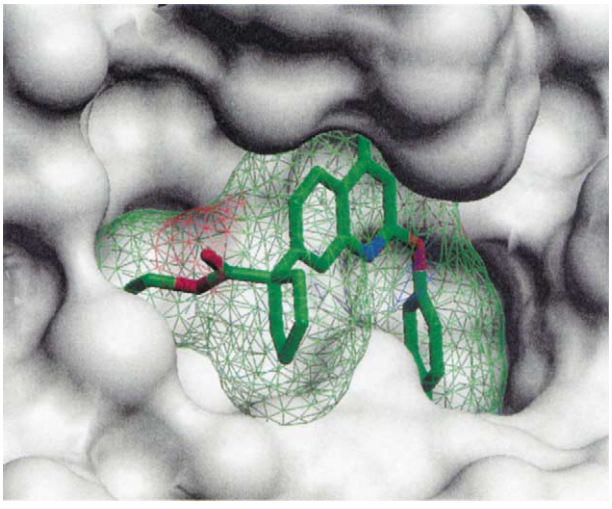
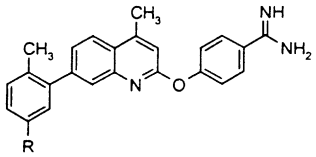


Figure 2. X-ray structure of the thrombin/5a complex.

Table 2. Thrombin inhibition and antithrombotic activity in vitro



Compd	R	Thrombin inhibition IC_{50}, μM	aPTT ED_{200}, μM	LogP (octanol/water)
3a	H	0.006	7.9	> 3.5
3d	$CONHCH_2CO_2Et$	0.010	3.3	1.3
3e	$CONHCH_2CO_2H$	0.008	0.9	0.5

with thrombin (Fig. 2). Surprisingly, the cyclopentyl group of **5a**, instead of fitting into the lipophilic S4-pocket, interacts with the lipophilic groove in front of the S4 subsite. As a consequence the ethyl ester points into the S4 subsite indicating that **5a** uses these two unexpected interactions which results in the best thrombin inhibition within this series of compounds. In addition, **5a** was the only example which also showed a weak inhibition of factor Xa ($IC_{50} = 1.5$ μM). The binding mode observed in thrombin also seemed to be very important for the inhibition of factor Xa and turned out to be the basis for the design of potent dual inhibitors of both coagulation enzymes.[16]

In order to reduce the high lipophilicity and to increase the antithrombotic potency in vitro we introduced additional hydrophilic groups. As indicated by the X-ray structure of **3c** in thrombin (Fig. 1) the C-5 carbon of the *o*-tolyl residue points into the water phase and offers the possibility of introducing polar substituents. The results of these modifications are shown in Table 2. As expected the introduction of ester or amide moieties at C-5 had no influence on enzyme inhibition (**3d**,**3e**). In contrast the antithrombotic potency in human plasma measured by the activated partial thromboplastin time (aPTT) was strongly affected and correlated very well with lipophilicity (log P octanol/water). The compound with the highest hydrophilicity (**3e**) was also the most potent example within this series. However, similar to other quinoline derivatives, **3e** showed only short duration of action after iv-administration to rats.

The quinoline derivatives described in this paper represent a new class of potent and selective thrombin inhibitors with remarkably low molecular weight (M_W: 335–391). Due to their high lipophilicity these compounds showed only modest antithrombotic activity in vitro which prevents their therapeutic use. By co-crystallization of two quinoline derivatives with thrombin we gained new knowledge concerning enzyme-inhibitor interactions, which are the structural basis for the further design of improved coagulation inhibitors. The progress we made with modified heterocyclic dual inhibitors of thrombin and factor Xa is reported in the following paper.[16]

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