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1-AMINOISOQUINOLINE AS BENZAMIDINE ISOSTER IN THE DESIGN AND SYNTHESIS OF ORALLY ACTIVE THROMBIN INHIBITORS

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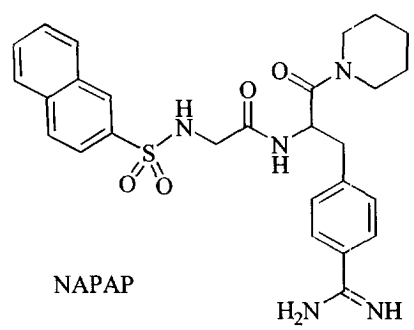
Abstract: Replacement of the highly basic benzamidine moiety of NAPAP by the moderately basic 1-aminoisoquinoline moiety resulted in thrombin inhibitors with improved selectivity towards trypsin and enhanced Caco-2 cell permeability

Introduction

Thrombin, a serine protease, plays a central role in the initiation and propagation of thrombotic events. It is well recognised that inhibitors of thrombin are potential anticoagulants. A potent reversible inhibitor of thrombin is *N*α-(2-naphthylsulfonylglycyl)-*p*-amidinophenyl-alanyl-piperidine (NAPAP, K_i = 6 nM).[1] The pharmacological profile of NAPAP is, apart from its antithrombotic activity, unattractive as it shows poor bioavailability after oral administration, while displaying a short half-life.[1,2] It has been suggested that the poor oral bioavailability of NAPAP is associated with the presence of the highly basic benzamidine moiety (pK_a ~ 12).[1] Furthermore, benzamidine based analogues of NAPAP in which the 2-naphthylsulfonylglycyl moiety was replaced by the 4-methoxy-2,3,6-trimethylphenylsulfonylaspartyl moiety and derivatives thereof with substitution at the β-carboxylic group of the aspartate showed low oral bioavailability in rat and low permeability across Caco-2 monolayers, a model for intestinal absorption.[3]



NAPAP

Replacement of NAPAP's benzamidine moiety however has shown to be accompanied by a strong decrease in potency. For instance Stürzebecher et al. replaced the amidino functionality of NAPAP by the less

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basic amino, cyano and oxamidino functionalities and found that the antithrombotic activity reduced considerably.[4] Replacement of NAPAP's amidino functionality by an amidrazone functionality led to a 600-fold reduction of thrombin inhibition.[5] Also replacement of NAPAP's benzamidine moiety by 4-aminopyridine (pK_a = 9.2) resulted in a decreased anti-thrombin activity.[6] Despite the limited success of replacing NAPAP's benzamidine moiety we were charmed by NAPAP's anti-thrombin potency and still were eager to mimic NAPAP's benzamidine moiety by isosters of reduced basicity. We reasoned that such isosters on the one hand should have reduced basicity in order to facilitate membrane permeability, while on the other hand the pK_a should be high enough to allow for a (partial) ionic interaction with Asp^{189} of thrombin. Thus, an optimal pK_a of the isoster would presumably be around eight. Furthermore, the isosters should maintain the three-point interaction as observed in the crystal structure of the NAPAP–thrombin complex,[7] have the correct (coplanar) direction of the hydrogen bonds, and display optimal van der Waals contacts with the S1 pocket of thrombin. Of the possibilities considered, 1-aminoisoquinoline (pKa = 7.5)[8] was the best candidate that fulfils these criteria (Figure 1).

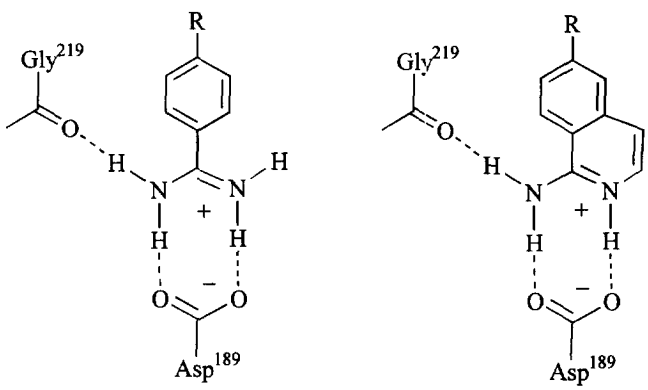
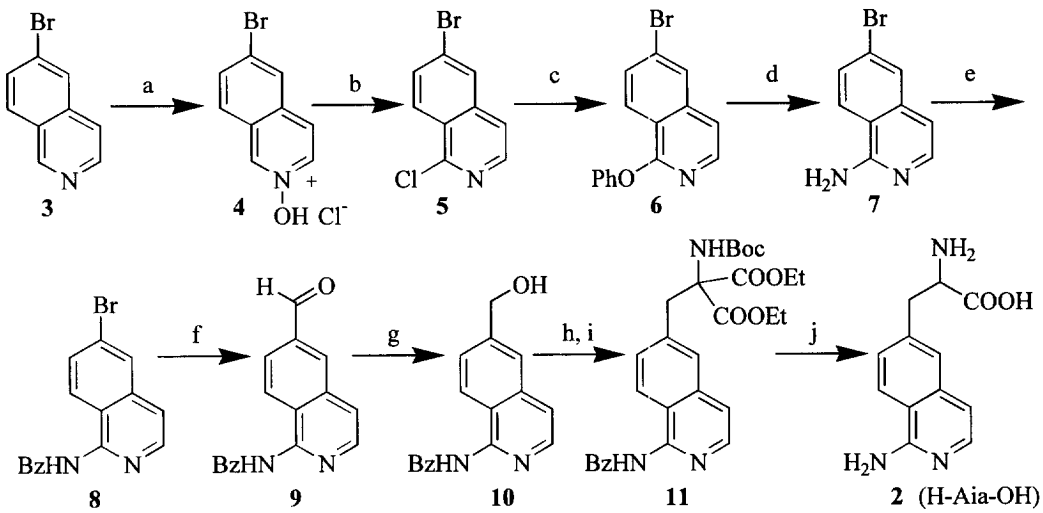


Figure 1. On the left a schematic drawing of the binding of NAPAP's benzamidine moiety to the S1 pocket of thrombin and on the right the corresponding putative binding mode of an 1-aminoisoquinoline group as benzamidine isoster.

Results and Discussion

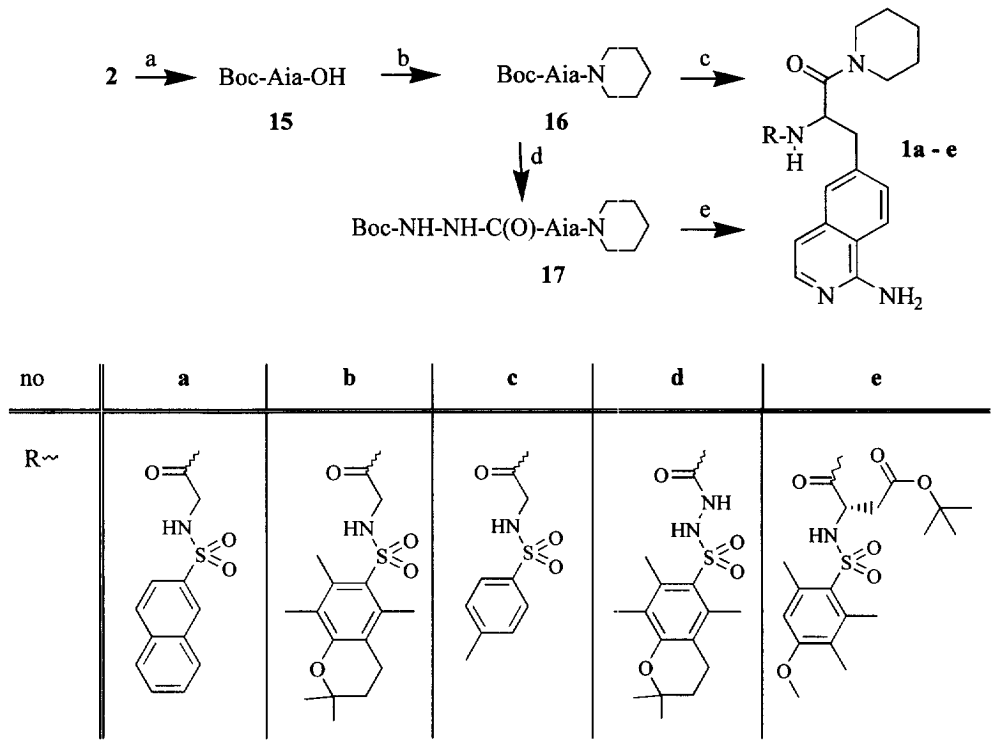
The amino acid required in the synthesis of 1-aminoisoquinoline based NAPAP-like compounds **1a–e** (see Scheme 2) is 2-amino-3-(6-[1-aminoisoquinolinyl])propionic acid **2** (= [6-(1-aminoisoquinolinyl)]alanine: H-Aia-OH, Scheme 1). This amino acid was prepared from 6-bromoisoquinoline **3** (Scheme 1).[9] Introduction of the amino functionality at position-1 of 6-bromoisoquinoline **3** using the Chichibabin reaction was not successful. For this reason we had to explore the long route of oxidation, introduction of chlorine at position-1, substitution by phenoxide and finally introduction of the amino functionality to give 1-amino-6-bromoisoquinoline **7**. However, various attempts to perform a Heck reaction on 1-amino-6-bromoisoquinoline **7**, its N-protected analogue benzamide **8** or its precursors phenoxide **5** and chloride **6** using methyl 2-

acetamidoacrylate failed. To prepare the desired amino acid **2** a long classic route had to be used. The amino functionality of bromide **7** was first protected as benzoyl amide. Trans-metalation using an excess of *n*-butyllithium and quenching with *N,N*-dimethylformamide (DMF) yielded aldehyde **9**. Reduction of this aldehyde, subsequent transformation of the resulting alcohol into a chloride and substitution of the chloride by a protected aminomalonate afforded malonate **11**. Finally, hydrolysis and decarboxylation yielded racemic amino acid **2** (H-Aia-OH).



Scheme 1. *Reagents and conditions:* (a) 1. *m*CPBA, 2. HCl, MeOH (96%). (b) $POCl_3$ (73%). (c) PhOH, KOH (99%). (d) Ammonium acetate (72%). (e) Benzoic anhydride (Bz_2O), pyridine (94%). (f) 1. THF, *n*-butyllithium (6 equiv), 2. DMF (60–70%). (g) THF, MeOH, $NaBH_4$ (99%). (h) 1. Methanesulfonyl chloride, CH_2Cl_2, Et_3N, 2. THF, LiCl. (i) Dioxane, EtOH, EtONa, $BocNHCH(COOEt)_2$ (56–68%). (j) AcOH, HCl, H_2O, 100 °C (99%).

Two routes were used to attach side chains to the new amino acid **2** (Scheme 2). Neither of these routes required the aryl amino functionality to be protected in the coupling reactions. The straight-forward route via step c was applied for the well-described *N*-(arylsulfonyl) amino acids. However, *N*-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)-α-aza-glycine was not described in literature, and therefore α-aza-glycine derivative **1d** was prepared via a somewhat longer route (Scheme 2, via step d and e) using the synthetic strategy and reagents described for the benzamidine counterpart of compound **1d**.[10]



Scheme 2. *Reagents and conditions:* (a) $(Boc)_2O$, Et_3N, MeOH (69%). (b) Piperidine, TBTU, DMF (88%). (c) 1. TFA, CH_2Cl_2, 2. R-OH, HOBt, *N*-ethylmorpholine, DCCI, DMF (61–82%). (d) 1. TFA, CH_2Cl_2, 2. BocNH-NH-C(O)-O-(*p*-nitrophenyl), *N*,*N*-diisopropylethylamine, DMF (88%). (e) 1. TFA, thioanisole, 2. 2,2,5,7,8-pentamethylchroman-6-sulfonyl chloride, *N*,*N*-diisopropylethylamine, DMF (52%).

The thrombin inhibition data of NAPAP and benzamidine isoster containing analogues are shown in Table 1. Comparison of NAPAP's anti-thrombin activity and that of its aminoisoquinoline counterpart **1a** shows that the activity is reduced considerably due to the modification of benzamidine into 1-aminoisoquinoline. Furthermore, comparison of the calculated K_i values of aminoisoquinolines **1b–1d** with the published K_i values of the corresponding benzamidine based compounds shows a reduction of anti-thrombin activity of the same magnitude. However, comparison of aminoisoquinoline **1e** (Org 37476) with its benzamidine counterpart shows a much more favourable behaviour since both analogues display an anti-thrombin activity of the same magnitude. The explanation for this rather remarkable activity is not clear at the moment and might be addressed by comparing high resolution structural data of thrombin–inhibitor complexes of the benzamidine and aminoisoquinoline based inhibitors. However, attempts to generate structural data of aminoisoquinoline based inhibitors in a complex with thrombin were not successful.

Table 1. The *in vitro* activity against thrombin and trypsin[11] and Caco-2 cell permeability (Papp).[12]

Compound	Thrombin IC_{50} (μM)	Thrombin [a] calc.K_i (nM)	Benzamidine counterpart[b] Thrombin K_i (nM)	Trypsin IC_{50} (μM)	Caco-2 Papp (nm/sec)
NAPAP	0.69	11		0.169	4
1a	55	860	6–14 (D,L) (= NAPAP)	255	19
1b	28	440	1.3 (D)	ND	37
1c	357	5500	48 (D,L)	ND	ND
1d	1.1	18	0.09 (D)	ND	ND
1e	0.082	1.3	0.9 (D)	378	50

[a] calc.K_i is the K_i value calculated from the determined IC_{50} value using the correlation found between the determined IC_{50} values and the determined K_i values of several competitive inhibitors.[11]

[b] K_i value for thrombin of the benzamidine counterpart reported in literature.[1, 10, 13, 14] (D,L) means K_i value of racemic benzamidine counterpart and (D) means K_i value of D-isomer of benzamidine counterpart.

ND: not determined

Compared with NAPAP the selectivity of compound **1a** against trypsin has been improved. What's more, the potent thrombin inhibitor **1e** has an excellent selectivity against trypsin. This selectivity might be due to the exchange of one amino acid in the S1 pockets of these proteases. Amino acid Ala^{190} of thrombin is Ser^{190} in trypsin and this makes the trypsin S1 pocket more polar and slightly smaller. Going from benzamidine to the more hydrophobic and bulkier 1-aminoisoquinoline will therefore be less favourable for the interaction with trypsin.

In literature Caco-2 cell monolayers are used as a model for intestinal absorption.[3, 15] Most compounds that show good absorption by passive diffusion across the intestinal membrane have a Caco-2 cell permeability (Papp) of around hundred nm/sec or larger.[16] As we anticipated, the highly basic NAPAP does not diffuse through the monolayer, whereas the less basic benzamidine isoster based compounds show enhanced permeability.

In conclusion, replacement of the highly basic benzamidine moiety of NAPAP by 1-aminoisoquinoline yielded thrombin inhibitors with enhanced Caco-2 cell permeability. In addition the selectivity towards trypsin improved considerably. In particularly compound **1e** is a potent (nM) inhibitor of thrombin, is highly selective towards trypsin and shows a reasonable Caco-2 cell permeability.[17]

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