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DESIGN, SYNTHESIS AND TESTING OF AMINO-BICYCLOARYL BASED ORALLY BIOAVAILABLE THROMBIN INHIBITORS

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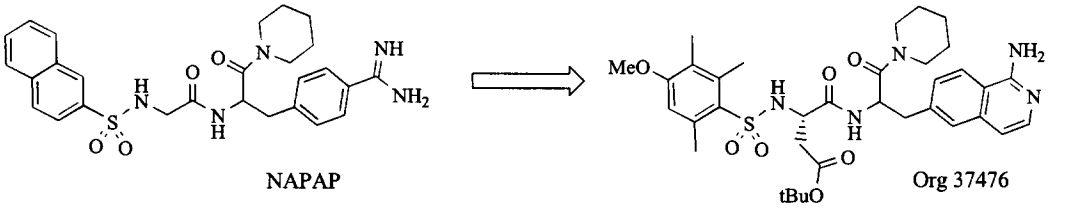
Abstract: Replacement of the highly basic benzamidine moiety with moderate basic amino-bicycloaryl moieties in a series of thrombin inhibitors related to NAPAMP provided potent enzyme inhibition and significant improvements in membrane transport and oral bioavailability.

Introduction

Thrombin plays a key role in the control of thrombus formation, for which reason its inhibition has become a target for new anticoagulants.[1] Important issues in the development of direct thrombin inhibitors are: potency, selectivity, oral bioavailability, and half-life in the circulatory system.[2] Although many direct inhibitors of thrombin have been discovered, most of these inhibitors lack sufficient oral bioavailability.[1] This poor oral bioavailability is often associated with the presence of highly basic functionalities such as guanidine and amidine. Recently, the replacement of the highly basic benzamidine moiety ($pK_a \sim 12$) of NAPAP (*N*α-(2-naphthylsulfonylglycyl)-4-amidinophenyl-alanyl-piperidine) by the less basic 1-amino-isoquinoline moiety ($pK_a = 7.5$) was described.[3] This replacement, combined with a limited optimisation effort, resulted in the potent and selective thrombin inhibitor Org 37476, which showed a relatively good permeability across Caco-2 cell monolayers, a model for intestinal absorption (Figure 1).



	Thrombin		Trypsin	Caco-2
	IC_{50} (μM)	K_i (nM)	IC_{50} (μM)	P_{app} (nm/s)
NAPAP	0.69	6–14	0.17	4
Org 37476	0.08		378	50

Figure 1. Structures and *in vitro* activities of NAPAP and Org 37476.[4]

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Since the use of 1-aminoisoquinoline as isoster of benzamidine worked well for NAPAP-like compounds, we wanted to apply this concept to the benzamidine-based thrombin inhibitor NAPAMP (*N*α-naphthylsulphonyl-3-amidinophenylalanyl-4-methylpiperidine).[5] Chemical intuition and modelling studies suggested that 6-substituted 1-aminoisoquinoline (6Aiq) would not be a good isoster for the 3-substituted benzamidine of NAPAMP-like compounds but that 7-substituted 1-aminoisoquinoline (7Aiq), 2-substituted 4-aminothieno[3,2-*c*]pyridine (Atp), and 2-substituted 4-aminofuro[3,2-*c*]pyridine (Afp) would be better suited as isosters (Figure 2). This paper describes the synthesis, the antithrombin activity, selectivity towards trypsin, Caco-2 cell permeability, and oral bioavailability of NAPAMP-like compounds containing these four amino-bicycloaryl moieties.

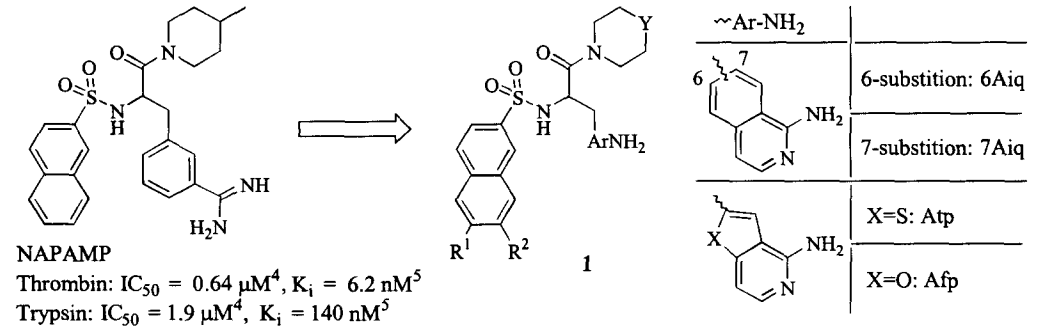
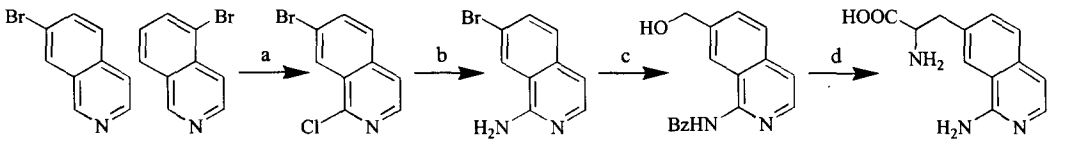


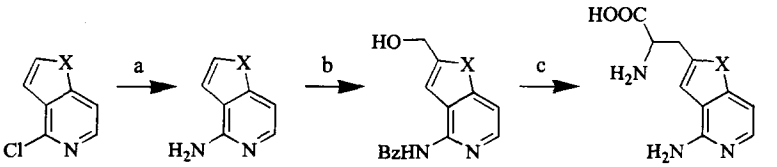
Figure 2. Structures and *in vitro* activities of NAPAMP[5] and putative benzamidine isosters.

Chemistry

In the syntheses of the heterocyclic-based analogues of NAPAMP the β-(amino-bicycloaryl)-alanines **6** constitute the central building blocks. The strategy described for the preparation of β-[6-(1-aminoisoquinoline)]alanine[3] was used for the preparation of β-[7-(1-aminoisoquinoline)]alanine (**6a**, Scheme 1). The starting material 7-bromoisoquinoline was synthesised from 3-bromobenzaldehyde. According to literature 7-bromoisoquinoline should be the major product.[6] However, we observed no selectivity, and 7-



Scheme 1. *Reagents and conditions:* (a) 1. *m*CPBA, CH_2Cl_2, room temperature (r.t.), 1h, 2. HCl, MeOH, 0 °C, 3. $POCl_3$, 90 °C, 2h (27%). (b) 1. PhOH, KOH, 140 °C, 2 h, 2. Ammonium acetate (NH_4OAc), 150 °C, 14 h (66%). (c) 1. Benzoic anhydride (Bz_2O), pyridine, 125 °C, 1 h, 2. THF, *n*-butyllithium (6 equiv.), -78 °C, 30 min, 3. DMF, 4. THF, MeOH, $NaBH_4$, r.t., 5 min (50%). (d) 1. Methanesulfonyl chloride (MsCl), CH_2Cl_2, Et_3N, r.t., 2 h, 2. THF, LiCl, r.t., 16 h, 3. Dioxane, EtOH, EtONa, $BocNHCH(COOEt)_2$, 80 °C, 2 h, 4. AcOH, HCl, H_2O, 100 °C, 16 h (74%).

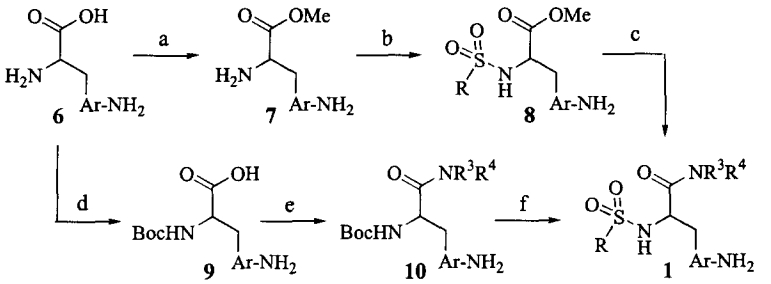


Scheme 2. *Reagents and conditions:* (a) 1. PhOH, KOH, 140 °C, 2 h, 2. NH_4OAc, 155 °C, 3 days. (b) 1. Bz_2O, pyridine, 125–160 °C, 2 h, 2. THF, -78 °C, X=S: LDA (2.3 equiv.), X=O: *n*-butyllithium (6.7 equiv.), 3. DMF, 4. THF, MeOH, $NaBH_4$, r.t., 5 min. (c) 1. MsCl, CH_2Cl_2, Et_3N, r.t., 2 h, 2. THF, LiCl, r.t., 16 h, 3. Dioxane, EtOH, EtONa, $BocNHCH(COOEt)_2$, 80 °C, 2 h, 4. AcOH, HCl, H_2O, 100 °C, 16 h.

bromoisoquinoline and its isomer 5-bromoisoquinoline were formed in almost equal amounts. These isomers were separated in the 1-chloroisoquinoline stage using column chromatography, and the resulting pure compound **3a** was transformed into racemic β-[7-(1-aminoisoquinoline)]alanine (**6a**).

A strategy similar to the one mentioned above was followed to prepare β-[2-(4-aminothieno[3,2-*c*]pyridine)]alanine (**6b**) and β-[2-(4-aminofuro[3,2-*c*]pyridine)]alanine (**6c**) (Scheme 2). Formylation of position 2 of 4-aminothieno[3,2-*c*]pyridine and 4-aminofuro[3,2-*c*]pyridine did not require metal halogen exchange as was the case with the isoquinolines but was accomplished by treating the protected heterocyles with a strong base followed by addition of N,N-dimethylformamide (DMF) to give aldehydes. Subsequent reduction gave alcohols **5b** and **5c** (Scheme 2, step b). The latter two compounds were converted into the racemic amino acids **6b** and **6c** using the same procedures as applied to the isoquinolines.

Two routes were used to transform the β-(amino-bicycloaryl)-alanines **6** into the desired end-products **1** (Scheme 3).[7] Neither of these routes required the aryl amino functionality to be protected.

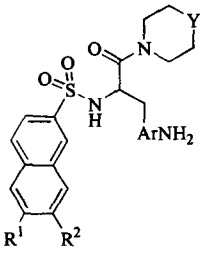


Scheme 3. *Reagents and conditions:* (a) MeOH, $SOCl_2$, 50 °C, 2 h (100%). (b) RSO_2Cl, Et_3N, CH_2Cl_2, 0 °C–r.t., 1 h (42–84%). (c) 1. NaOH, water, dioxane, r.t. or NaOH, water, MeOH, THF, r.t., 2. HNR^3R^4, TBTU, DMF, r.t., 16 h (12–95%). (d) Boc_2O, Et_3N, MeOH, r.t. (100%). (e) HNR^3R^4, TBTU, DMF, r.t., 1h (77–84%). (f) 1. TFA/CH_2Cl_2 = 1/1, r.t., 1 h, 2. RSO_2Cl, Et_3N, CH_2Cl_2, 0 °C–r.t., 1 h (49–78%).

Biological Activity

Replacement of the benzamidine moiety of NAPAMP by 4-aminothieno[3,2-*c*]pyridine resulted in a 400-fold reduction of thrombin inhibitory potency but an excellent Caco-2 permeability was obtained (Table 1, compound **1a**). In the exploration of 1-aminoisoquinoline as benzamidine isoster in NAPAP, a limited structure–activity relationship (SAR) study was required to establish the potential of this isoster.[3] NAPAMP was therefore approached in the same way, and a limited series of analogues of amino-thieno[3,2-*c*]pyridine **1a** was prepared. The data of SAR studies reported for NAPAMP and Argatroban, diplaying similar binding modes with thrombin, served as inspiration in the design of analogues of compound **1a**. Introduction of a methoxy group at position 7 of the naphthyl moiety of compound **1a** remarkably enhanced the antithrombin activity and the excellent Caco-2 permeability was maintained (Table 1, compound **1c**). Modifications of the methylpiperidinyl moiety gave compounds that displayed a similar or lower potency. In addition, the 6 and 7- substituted 1-amino-isoquinoline and 2-substituted 4-aminofuro[3,2-*c*]pyridine analogues of compound **1c** were prepared. From this series, 7-substituted 1-aminoisoquinoline **1g** showed a thrombin inhibition similar to aminothieno[3,2-*c*]pyridine **1c**. These inhibitors both display a thrombin inhibitory activity and a selectivity towards trypsin similar to NAPAMP itself. Furo[3,2-*c*]pyridine **1i** was slightly less potent, and as expected 6-substituted 1-aminoisoquinoline **1h** showed only modest thrombin inhibition. As a result, thieno[3,2-*c*]pyridine **1c** was selected for administration to dogs and its oral bioavailability in dogs turned out to be 36%.[8]

Table 1. *In vitro* activities against thrombin, trypsin and Caco-2 cell permeability of compounds **1**.[4]



no	R^1	R^2	$ArNH_2$	Y	Thrombin IC_{50} (μM)	Trypsin IC_{50} (μM)	Caco-2 P_{app} (nm/s)
1a	H	H	Atp	CHMe	209	205	117
1b	OMe	OMe	Atp	CHMe	5	4	97
1c	H	OMe	Atp	CHMe	0.53	4	121
1d	H	OMe	Atp	NSO_2Me	0.56	18	10
1e	H	OMe	Atp	NMe	6	13	22
1f	H	OMe	Atp	CHC(O)Me	1.4	2	23
1g	H	OMe	7Aiq	CHMe	0.63	8	53
1h	H	OMe	6Aiq	CHMe	48	27	75
1i	H	OMe	Afp	CHMe	1.58	6	9

The group of thrombin inhibitors like NAPAMP and compounds **1** can broadly be characterised as inhibitors in which the carboxylate of the central amino acid is functionalised as a tertiary amide and the α-position is substituted with an aryl sulfonamide moiety. Within this group some moderate to good thrombin inhibitors with good intestinal absorption (demonstrated by good Caco-2 cell permeability or good oral bioavailability) have been disclosed in which the central amino acid contains a heteroaryl moiety of low basicity such as: the aminopyridine,[9] benzamidrazone,[10] benzylamine,[11] and aminobenzene.[12] However, none of these heterocycles incorporated into NAPAP-like compounds yielded potent thrombin inhibitors with good intestinal absorption. In our case, the concept of using amino-bicycloaryl moieties as benzamidine isoster worked well both in the NAPAP-type of inhibitors[3] (Figure 1) and in the NAPAMP-type of inhibitors as demonstrated by potent thrombin inhibition in combination with the excellent Caco-2 cell permeability. These findings clearly illustrate the value of this concept and additional research is performed to evaluate it in other classes of benzamidine based inhibitors.

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References and Notes

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7. Compounds **1** were characterised by NMR, MS, IC and HPLC.
8. The oral bioavailability of compound **1c** (racemate) in dogs was studied in Beagle dogs (weighing approximately 20 kg) which were given compound **1c** at a dose of 10 mg/kg in 5% Gummi arabicum orally (n = 2) or in PEG 400/saline = 1/1 intravenously (n = 1). Plasma samples were collected and the

concentrations of compound **1c** were determined using HPLC. The enantiomeric ratio of **1c** in the plasma samples was not determined. The individual oral bioavailability in these dogs was 31% and 41%.

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