Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2837–2842

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

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

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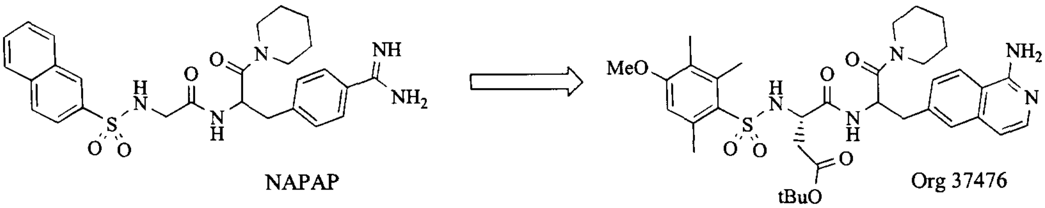
Received 6 July 1999; accepted 30 August 1999

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).



NAPAP

Org 37476

	Thrombin IC_{50} (μM)	Thrombin K_i (nM)	Trypsin IC_{50} (μM)	Caco-2 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]

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00483-7

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.

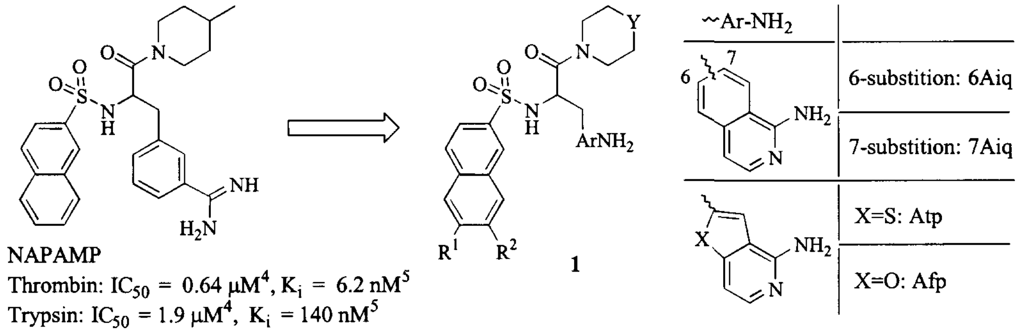
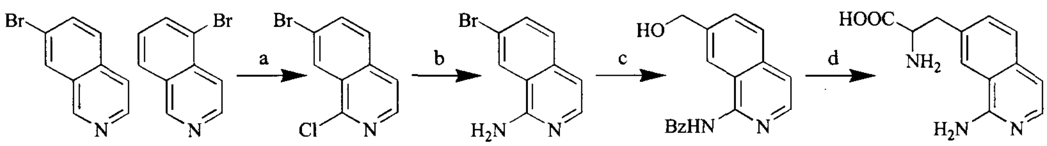


Figure 2. Structures and *in vitro* activities of NAPAMP[5] and putative benzamidine isosters.

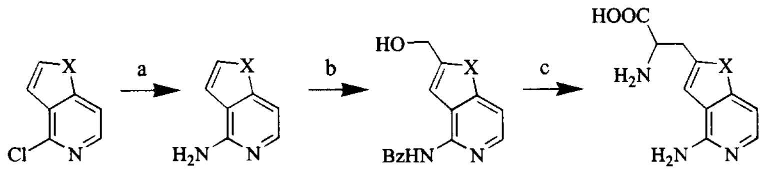
Chemistry

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-



2a (mixture of isomers) **3a** **4a** **5a** **6a**

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%).



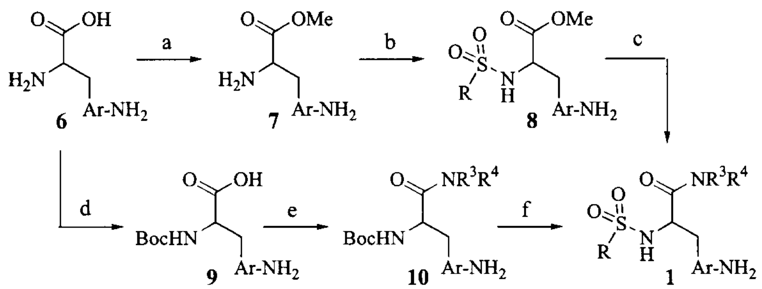
3b, X=S **4b**, X=S: 73% **5b**, X=S: 91% **6b**, X=S: 47-61%
3c, X=O **4c**, X=O: 45% **5c**, X=O: 39% **6c**, X=O: 16%

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.

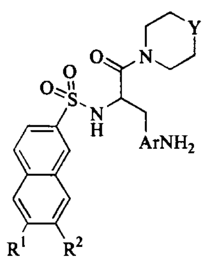


Scheme 3. *Reagents and conditions:* (a) MeOH, $SOCl_2$, 50 °C, 2 h (100%). (b) RSO_2Cl, Et_3N, CH_2Cl_2, 0 °C–r.t., 1 h (42–84%). (c) 1. NaOH, water, dioxane, r.t. or NaOH, water, MeOH, THF, r.t., 2. HNR^3R^4, TBTU, DMF, r.t., 16 h (12–95%). (d) Boc_2O, Et_3N, MeOH, r.t. (100%). (e) HNR^3R^4, TBTU,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

Acknowledgement: We would like to thank Ms. Y. Diepeveen for chemical characterisation, Ms. M. van der Pol for analytical support and Dr. K. Stegmeier of Roche (Mannheim, Germany) for performing the bioavailability studies in dogs.

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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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