Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2017–2021

Investigation of mechanism-based thrombin inhibitors: Implications of a highly conserved water molecule for the binding of coumarins within the S pocket

Raphaël Frédérick,[a,†] Caroline Charlier,[b,†] Séverine Robert,[a] Johan Wouters,[b] Bernard Masereel[a] and Lionel Pochet[a,*]

[a]*Department of Pharmacy, Drug Design and Discovery Center, University of Namur, FUNDP, 61, rue de Bruxelles, B-5000 Namur, Belgium*
[b]*Department of Chemistry, Drug Design and Discovery Center, University of Namur, FUNDP, 61, rue de Bruxelles, B-5000 Namur, Belgium*

Received 4 November 2005; revised 19 December 2005; accepted 19 December 2005
Available online 18 January 2006

Abstract—The synthesis of novel coumarins bearing on the lateral side chain in the 3-position an amine or a guanidine group is described. In vitro evaluation highlighted **14d** which possesses a *meta* aniline side chain as a very potent THR inhibitor. Surprisingly, the introduction of a guanidine moiety always led to a decrease in THR inhibiting properties. We, thus, used docking experiments to rationalize the SAR in the series. This study showed the crucial role of a conserved water molecule in the specificity pocket of THR during docking simulation in order to explain the inactivity of guanidine derivatives.

Thrombin (THR) is a trypsin-like serine protease which plays a pivotal role in the process of haemostasis and thrombosis. Besides exerting multifunctional activities in the coagulation cascade, it is also one of the main activators of platelet secretion and aggregation.[1,2] So, THR has earlier been recognized as a key target for the development of new antithrombotics.

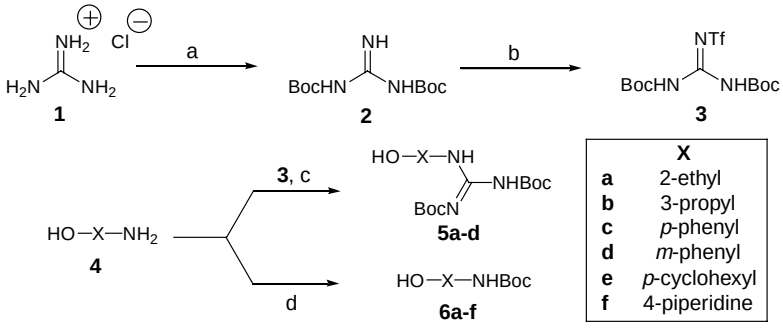
As part of a project aiming at the development of coumarins as selective serine protease inhibitors, we have recently described a series of coumarins as THR and factor Xa (FXa) inhibitors.[3] They are characterized by a chloromethyl moiety in the 6-position and a hydrophobic alkyl-, aryl-, heteroaryl-ester, amide or thioster in the 3-position. These compounds were found to act as mechanism-based inhibitors. The first step of their inhibition mechanism consists in the nucleophilic attack by the activated hydroxyl group of Ser195 on the lactone moiety, leading to the acyl-enzyme. Then, the departure of the chlorine atom promotes the formation of a highly reactive quinone methide which could be alkylated and thus leads to the irreversible inactivation of the enzyme.

In the present report, we investigated the synthesis and structure–activity relationships (SAR) of novel coumarins bearing an amine or a guanidine on the ester lateral side chain in the 3-position. These basic moieties could advantageously interact with the negatively charged Asp189 in the specificity (S) pocket. This strategy should therefore lead to new potent THR inhibitors. Pyridinic derivatives have been also investigated.

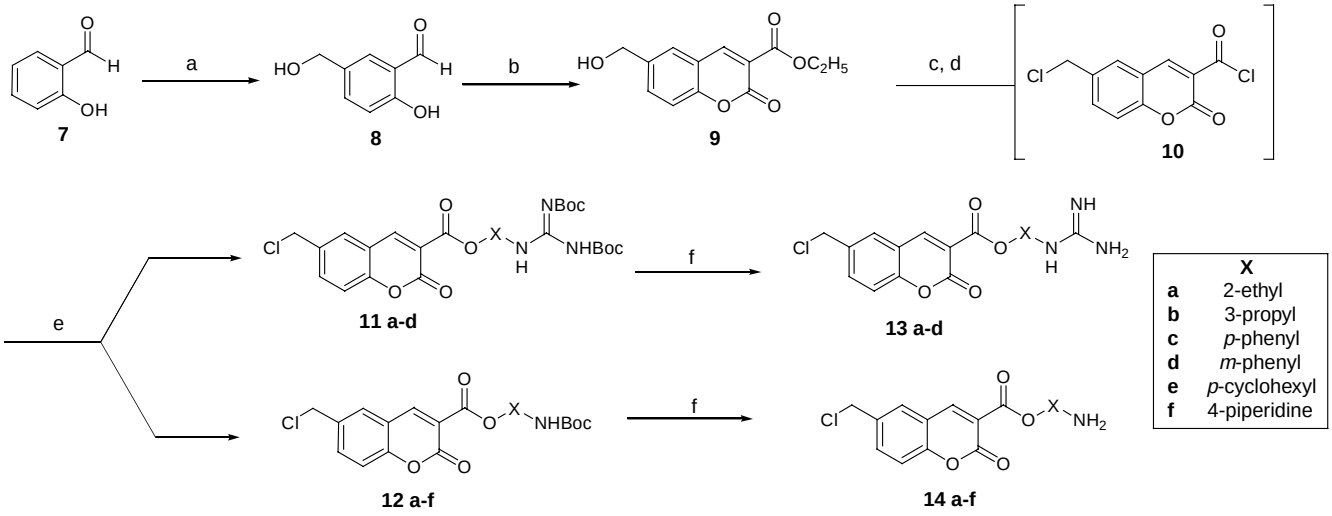
The synthetic routes of the newly designed coumarins are depicted on Schemes 1 and 2. The introduction of the ester lateral side chains on the 3-position needs the preparation of the protected guanidino (**5a**–**d**) and amino alcohols (**6a**–**f**). Among the various methods developed to prepare Boc-protected guanidines,[4,5] we chose the *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*″-triflylguanidine **3** which allows to access the alcohols (**5a**–**d**) in one step, starting from the commercially available amino derivatives **4**.[6,7] The intermediate **3** was easily obtained from the guanidinium hydrochloride **1**, by reaction with di-

Keywords: Thrombin; Coumarins; Docking; Serine protease; Mechanism-based inhibitor.
* Corresponding author. Tel.: +32 81 72 42 92; fax: +32 81 72 42 99; e-mail: lionel.pochet@fundp.ac.be
† These authors contributed equally to this work.

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.12.070



Scheme 1. Reagents and conditions: (a) Boc_2O, NaOH, 0 °C; (b) Tf_2O, Et_3N, −78 °C; (c) CH_2Cl_2, rt; (d) Boc_2O, dioxane, rt, 2 h.



Scheme 2. Reagents and conditions: (a) HCOH, HCl, 80 °C, 20 min; (b) diethyl malonate, piperidine, AcOH, EtOH, reflux, 17 h; (c) HCl (3 N), EtOH, reflux, 3 h; (d) $SOCl_2$, reflux, 3 h; (e) **5a–d** or **6a–f**, dioxane, pyridine, rt, 2 h; (f) TFA, CH_2Cl_2 (1:1), rt, 10 min.

tert-butyl-dicarbonate and sodium hydroxide at 0 °C followed by reaction at −78 °C with triflic anhydride and Et_3N (Scheme 1).[8] The Boc-protected amino alcohols (**6a–f**) were synthesized by reaction of the hydroxyl-amino (**4a–f**) with di-*tert*-butyl-dicarbonate.

Then, the suitable *N*-protected alcohol was reacted with the acyl halide (**10**), obtained by a previously described procedure,[9] to afford (**11a–d**) and (**12a–f**) (Scheme 2).[8] Finally, deprotection of derivatives (**11a–d**) and (**12a–d**) led to the targeted guanidines (**13a–d**) and amines (**14a–f**).[8]

Pyridinic compounds **15a–e** were obtained according to our previously published procedure.[9,10]

Table 1 summarizes the THR inhibitory potency of the newly synthesized compounds.[11] Surprisingly, guanidine derivatives (**13a–d**) are found to be almost inactive. Only **13a** and **13c**, bearing, respectively, a 2-guanidino-ethyl and a 4-guanidino-phenyl as side chain in the 3-position, possess a weak THR inhibitory potency. On the contrary, in the amino series, potent THR inactivators are obtained when the amine group is introduced on a phenyl ring (**14c–d**), particularly in the *meta*-position (**14d**). However, the introduction of an amine moiety on linear (**14a–b**) or cyclic (**14e–f**) aliphatic side chains always leads to a decrease in THR inhibitory potency. Compound **14d**, which possesses a *meta*-aminophenyl as side chain in the 3-position, is the best compound in this series with an IC_{50} value of 1.98 μM. For comparison purpose, we determined their kinetic parameters k_i and K_I (Table 2).[12] **14d** possesses a k_i/K_I ratio equal to 3570 M^{-1} s^{-1} and thus appears to be about 10-fold less potent than **16**, the best THR inhibitor of our previous study.[3] Moreover, the introduction of basic moieties such as guanidine, which proved to be very efficient on other series,[13] leads to an important decrease in THR inhibitory potency.

In the pyridine series (**15a–e**), only derivatives possessing the pyridine nitrogen in the 2′-position (**15a–b**) inhibit THR and particularly when a chlorine atom is located in the 3′-position.

With a view to helping in the understanding of these surprising results, we explored the binding mode of representative compounds within the THR active site by means of molecular modelling. Derivatives **13c**, **13d**, **14c** and **14d** were docked into the THR cavity (from