Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 45–49

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Molecular Design and Structure–Activity Relationships Leading to the Potent, Selective, and Orally Active Thrombin Active Site Inhibitor BMS-189664

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Received 16 July 2001; accepted 14 September 2001

**Abstract**—A series of structurally novel small molecule inhibitors of human α-thrombin was prepared to elucidate their structure–activity relationships (SARs), selectivity and activity in vivo. BMS-189664 (**3**) is identified as a potent, selective, and orally active reversible inhibitor of human α-thrombin which is efficacious in vivo in a mouse lethality model, and at inhibiting both arterial and venous thrombosis in cynomolgus monkey models. 

Development of direct acting thrombin inhibitors[1] has been an area of intense research over the past decades. In the accompanying communication[1] we outline our structure based drug design approach leading to a novel template as exemplified by **1** (Fig. 1). Further structural modification led to the identification of **2** as a highly potent, selective, and reversible thrombin active site inhibitor. In this communication, we describe studies based on the same template, that have culminated in the discovery of **3** as a potent, selective, and orally active thrombin inhibitor with excellent antithrombotic activity in vivo. The synthesis, SAR and biological studies leading to **3** (BMS-189664) are described briefly.

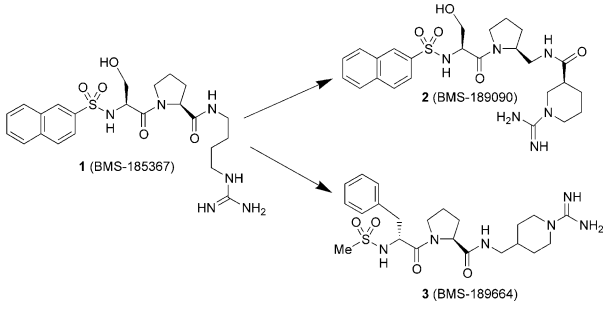
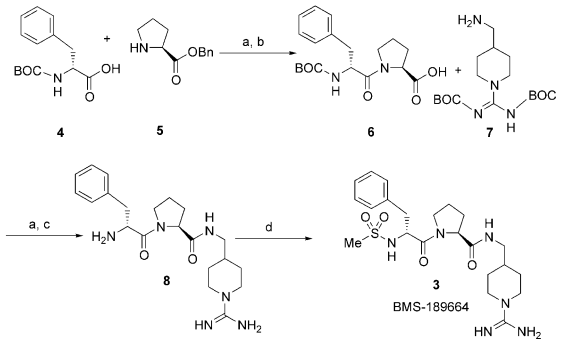



**Figure 1.** Schematic representation of the inhibitor design from **1**.

The synthesis of this class of compounds follows a general synthetic route that is illustrated in Scheme 1. Coupling of BOC-D-phenylalanine **4** and benzyl L-prolinate **5** under standard amide coupling conditions followed by hydrogenolysis of the benzyl ester afforded the acid **6**. The acid was coupled with bis-BOC-guanidine **7**[2]




**Scheme 1.** Synthesis of **3**: (a) EDAC, HOBt, DMF, NMM; (b) 10% Pd–C, $H_2$, EtOH; (c) $CH_2Cl_2$, $CF_3COOH$; (d) $CH_2Cl_2$, $Et_3N$, $CH_3SO_2Cl$.

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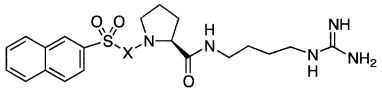
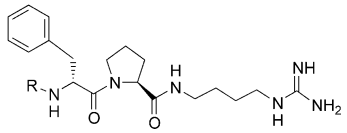
PII: S0960-894X(01)00667-9

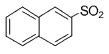
**Table 1.** SAR of amino acid modifications at $P_3$



| Compd | X | Thrombin inhibition $IC_{50}$ (μM)[3] | Compd | X | Thrombin inhibition $IC_{50}$ (μM)[3] |
|---|---|---|---|---|---|
| **9** | Gly | 2.9 | **14** | L-*allo*-Thr | 3.5 |
| **1** | L-Ser | 2.8 | **15** | L-Asp | 5.2 |
| **10** | L-Threo | 3.0 | **16** | D-Phe | 1.4 |
| **11** | L-Lys | 4.9 | **17** | D-Gln | 26.6 |
| **12** | L-Phe | 12.0 | **18** | D-Trp | 8.9 |
| **13** | L-Gln | 2.9 | **19** | D-Glu | > 333 |

**Table 2.** SAR from the sulfonamide modification at $P_3$



| Compd | R | Thrombin inhibition $IC_{50}$ (μM)[3] | Compd | R | Thrombin inhibition $IC_{50}$ (μM)[3] |
|---|---|---|---|---|---|
| **20** | H | 0.54 | **23** | $CH_3CO$ | 42 |
| **21** | $CH_3SO_2$ | 1.4 | **24** | $PhCH_2OCO$ | 0.67 |
| **22** | $CF_3SO_2$ | 110 | **16** |  | 1.4 |

under standard conditions to form an amide, which was treated with trifluoroacetic acid in dichloromethane to form amino-guanidine **8**. Reaction of **8** with methanesulfonyl chloride in dichloromethane in presence of triethyl amine afforded **3** (BMS-189664) after purification by reverse-phase preparative HPLC. This synthetic route is quite general and was used for the synthesis of other analogues in this series.

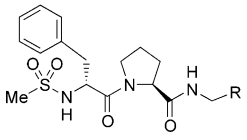
Compound **3** (BMS-189664) and its analogues were tested for their ability to inhibit thrombin hydrolysis[3] of the chromogenic substrate S-2238 ($IC_{50}$, Tables 1–5).

With **1** (BMS-185367) as a lead we studied the effect of amino acid substitution at $P_3$, summarized in Table 1. Most of the amino acid modifications (**1** and **10**–**18**) are well tolerated with minimal effect in thrombin inhibitory activity in vitro. In contrast, the D-amino acid Glu is detrimental to activity in vitro. Because of its potency, the D-Phe analogue **16** was selected for additional structure–activity studies.

Table 2 outlines the SAR observed with the modification of the sulfonamide functionality at $P_3$. The data demonstrate that the naphthalene sulfonamide group is not an absolute requirement for thrombin inhibitory activity. The corresponding amine **20** and the benzyl carbamate **24** are roughly 2-fold more potent while the methylsulfonamide **21** is equipotent to **16**. Both trifluoromethylsulfonamide (**22**) and acetamido (**24**) groups are not tolerated at this position.

In order to further optimize the potency, we investigated the modification of the guanidine moiety that binds to the Asp189 residue in the $P_1$ specificity pocket of thrombin (Table 3). Replacement of the guanidine group by an imidazole (**25**, and **27**) significantly reduced the potency, while a benzyl amine replacement (**26**) was

**Table 3.** SAR from the $P_1$–$P_2$ linker modification



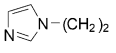
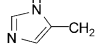
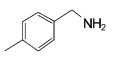
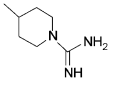
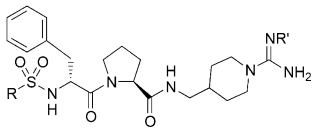
| Compd | R | Thrombin inhibition $IC_{50}$ (μM)[3] | Compd | R | Thrombin inhibition $IC_{50}$ (μM)[3] |
|---|---|---|---|---|---|
| **25** |  | 108 | **27** |  | 134 |
| **26** |  | 4.4 | **3** |  | 0.046 |

**Table 4.** SAR from the sulfonamide and guanidine modification




| Compd | R | R' | Thrombin inhibition $IC_{50}$ (μM)[3] | Mouse $ID_{50}$ (mpk[4] iv) | Mouse $ID_{50}$ (mpk[4] po) |
|---|---|---|---|---|---|
| **3** | Me | H | 0.046 | 0.19 | 8.8 |
| **28** | $PhCH_2$ | H | 0.01 | 0.24 | 23 |
| **29** | Me | OH | 0.07 | 0.17 | 10.5 |
| **30** | $PhCH_2$ | OH | 0.002 | 0.3 | 18 |

**Table 5.** Enzyme selectivity of selected thrombin inhibitors

| Compd | Thrombin $IC_{50}$ (μM)[4] | Trypsin (relative to thrombin)[a] | Plasmin (relative to thrombin)[a] | tPA (relative to thrombin)[a] | Factor Xa (relative to thrombin)[a] |
|---|---|---|---|---|---|
| **3** | 0.46 | 40 | 1000 | 2100 | > 7200 |
| **30** | 0.002 | 60 | 700 | 700 | 14,000 |
| Argatroban | 0.038 | 658 | 4900 | 6900 | > 1700 |
| Efegatran | 0.018 | 0.4 | 13,000 | 200 | 300 |

[a]Selectivity ratio: $IC_{50}/IC_{50}$ (thrombin).

tolerated. More importantly, introduction of a cyclic guanidine moiety (**3**) resulted in a 30-fold increase in potency, which we attribute to increased hydrophobic interactions of the piperidine ring with residues in the specificity pocket of thrombin.

Further optimization of **3** focused on modification of the sulfonamide and guanidine functionalities and is reported in Table 4. Replacement of the methylsulfonamide group with benzylsulfonamide (**28**) resulted in a 5-fold increase in potency, while the replacement of the guanidine residue of **3** by hydroxyguanidine (**29**) had minimal effect on potency. In contrast similar hydroxyguanidine replacement in the benzylsulfonamide analogue **28** resulted in a 5-fold increase in thrombin inhibitory activity (**30**, $IC_{50} = 2$ nM).

Compounds **3** and **28**–**30** were evaluated further for activity in vivo in a thrombin-induced mouse lethality model.[4] Compound **3** (BMS-189664) is the most potent analogue in this model in vivo when dosed by either the oral or intravenous route. Despite its superior potency in vitro, **30** is roughly 2-fold less potent than **3** in vivo. The in vivo oral activity of **3** (BMS-189664, $ID_{50} = 8.8$ mpk) is significantly superior to that of Efegatran ($ID_{50} = 23$ mpk) and Argatroban ($ID_{50} > 100$ mpk).

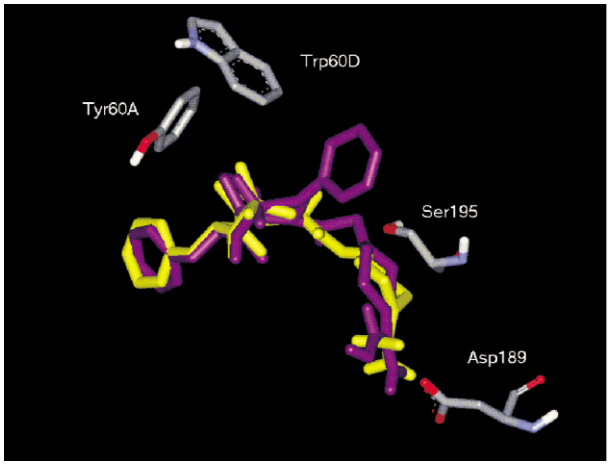



**Figure 2.** Superimposed conformations of **3** (yellow) and **30** (purple) bound to the α-thrombin/hirugen complex.

Separate studies[5] were performed to verify the $K_i$ of **3** and **30**. Compound **30** (BMS-191032) displayed classic competitive kinetics for thrombin inhibition with a $K_i$ of $460 \pm 35$ pM and was identified as the most potent thrombin inhibitor in this series. The $K_i$ of **3** (BMS-189664) determined under these conditions was $8.17 \pm 0.78$ nM, which is similar to 9.32 nM determined from $IC_{50}$.

The two most potent compounds in this series, **3** and **30**, were tested for their selectivity for thrombin over other serine proteases.[1] Both **3** and **30** displayed excellent selectivity for thrombin relative to other serine proteases important to hemostasis and thrombolysis (Table 5). The selectivity for thrombin over trypsin varies from 40- to 60-fold and the selectivities over plasmin and tPA vary from 700- to greater than 10,000-fold.

The crystal structures of the ternary complexes of human α-thrombin with hirugen and thrombin inhibitors **3** (BMS-189664) and **30** (BMS-191032) were determined at a resolution of 2.6 Å and 2.4 Å, respectively, by a procedure described earlier[6] (Fig. 2). Both inhibitors bind to thrombin in an antiparallel fashion. In this binding mode, the inhibitor backbone lies next to the extended thrombin backbone Ser214-Glu217 and forms a pair of hydrogen bonds to Gly216. This binding mode is very similar to the ones reported for Argatroban[7] and D-Phe-Pro-Arg$CH_2$Cl (PPACK).[8] The proline group of the inhibitors binds to the proximal P-pocket (also referred to as $S_2$ apolar hydrophobic pocket) and makes contact with Tyr60A and Trp60D. The two nitrogens of

**Table 6.** Single-dose pharmacokinetic parameters of BMS-189664

| Parameters | Monkeys | Beagle dogs | Parameters | Monkeys | Beagle dogs |
|---|---|---|---|---|---|
| IV Route | 13 | 12 | Oral route | 50 | 28 |
| Dose (μmol/kg) | ($n=3$) | ($n=7$) | Dose (μmol/kg) | ($n=3$) | ($n=7$) |
| Half-life (h) | 6.4 | 9[a] | $C_{max}$ (μM) | 5.6 | 3 |
| Clearance (mL/min/kg) | 6.4 | 13.4 | $T_{max}$ (h) | 2.3 | 0.6 |
| MRT (h) | 1.8 | 1.1 | MRT (h) | 6 | 4.0 |
| Vss (L/kg) | 0.7 | 0.8 | F (%) | 17 | 15 |

[a]Value derived from three dogs that had reliable terminal phase.

the guanidino moiety (**3**), are nestled in the specifity pocket and make hydrogen bonding contacts with Asp189. The hydroxyguanidine function in **30** binds in a very similar fashion. The D-phenylalanine moieties lie in the distal D-pocket formed by the side chains of Trp215, Ile174 and Glu97A-Leu99, while the methyl sulfonamido (**3**) or the benzylsulfonamido (**30**) groups cover the specificity pocket of the enzyme. The more pronounced thrombin inhibitory potency of **30** (23-fold more potent in vitro than **3**) may be attributed in part to the increased hydrophobic interactions between the benzyl group and the residues at the surface of the specificity pocket.

Because of its superior activity in vivo in the anesthetized mouse model BMS-189664 (**3**) was characterized further in vitro and in vivo. A modified thrombin time (TT) was used to determine the direct inhibition of thrombin activity in a protein-rich plasma environment. In this assay, BMS-189664 doubled thrombin clotting time in vitro at $51\pm9$ nM ($n=3$). In studies of human gel-filtered platelet aggregation stimulated by thrombin, BMS-189664 inhibited thrombin induced platelet aggregation with a p$A_2$ value of $8.43\pm0.04$ ($n=3$) with a slope near unity ($0.89\pm0.06$). In these studies, BMS-189664 is not likely to be acting as a thrombin receptor antagonist since it does not inhibit [$^3$H]SFFLRR-NH$_2$ binding ($IC_{50}>>100$ μM) to human platelet membranes. BMS-189664 also did not inhibit SFLLRNP-stimulated platelet aggregation.

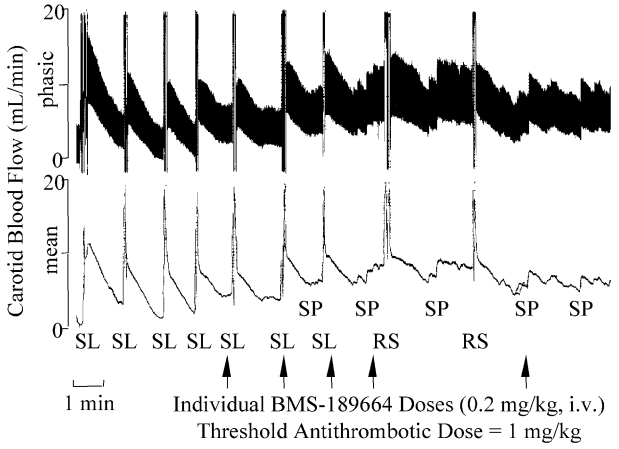



**Figure 3.** BMS-189664 inhibited arterial thrombosis in monkeys. Platelet-mediated reductions in blood flow were interrupted by momentary removal of a stenosis on the crush-injured artery. This shaking loose (SL) of the thrombus was required to prevent occlusion and resulted in a transient recovery of blood flow. The stenosis was then replaced to reinstate the cycle. BMS-189664 injections (0.2 mg/kg) were repeated until spontaneous blood flow restoration (SP) negated the need for SL to maintain stable blood flow. Once several SP were observed, the stenosis was reset (RS) in an attempt to reestablish the cyclic pattern. In this monkey a cumulative iv threshold dose of 1 mg/kg produced a persistent SP pattern.

The pharmacokinetic profile of BMS-189664 was determined in several animal species (Table 6). Single iv and oral doses of BMS-189664 were given to dogs and cynomolgus monkeys. BMS-189664 concentrations in plasma were determined using an LC/MS assay. Plasma concentrations declined with a mean elimination half-life of $>6$ h in both species. The oral bioavailability of BMS-189664 based on the dose-normalized AUCs after oral and iv dosing was 15 and 17% in dogs and monkeys, respectively.

In pentobarbital-anesthetized cynomolgus monkeys, BMS-189664 inhibited arterial and venous thrombosis at doses causing small increases in bleeding time and more pronounced prolongation of ex vivo clotting time. Thrombosis was induced in stenotic carotid arteries using the Folts model[9] as detailed in a representative experiment (Fig. 3). Arterial thrombosis was disrupted in six out of eight monkeys at a threshold dose of $1.5\pm0.2$ mg/kg (cumulative iv dose), which increased the APTT to $3.7\pm0.2$ times control. Vehicle was without effect in five monkeys. In other monkeys thrombosis was induced by topical application of filter paper saturated with 25% $FeCl_2$ for 3 min to a vena cava in which flow was impaired with a stenosis. Thrombus weight was reduced by 46, 88, and 90% by BMS-189664 doses of 0.5, 2, and 6 mg/kg, respectively (cumulative doses achieved through continuous iv infusions for 1 h), while

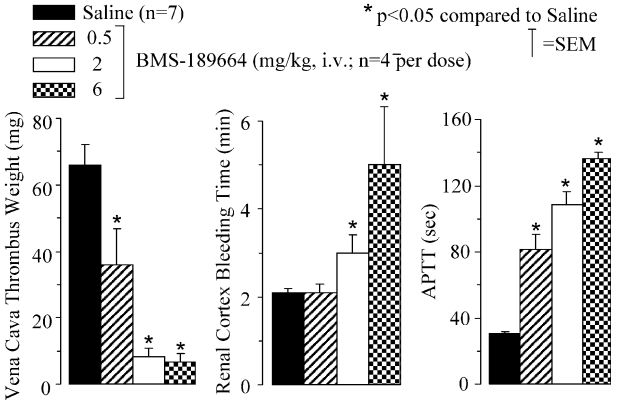



**Figure 4.** Dose-dependent effect of BMS-189664 on venous thrombosis, bleeding time and ex vivo clotting time was determined in anesthetized monkeys. BMS-189664 was infused at 9, 25, and 100 μg/kg/min for 1 h to achieve 0.5, 2, and 6 mg/kg, respectively.

the APTT was increased by 2.4, 3.2, and 4.3 times control, respectively (Fig. 4). Renal cortex bleeding time was also determined in these monkeys before and after thrombus formation using a Surgicutt® device (International Technidyne Corp., Edison, NJ, USA) to produce a 1-mm deep incision. The same BMS-189664 doses prolonged bleeding time by 8, 62, and 115%, respectively (Fig. 4). The APTT and bleeding time were not significantly affected in vehicle-treated monkeys.

In conclusion, we have described the SAR of a structurally novel series of compounds leading to BMS-189664 as a potent, highly selective, and orally active reversible thrombin inhibitor. In addition, we have identified BMS-191032 as the most potent thrombin inhibitor in vitro ($K_i = 460 \pm 35$ pM) in this series. The data provided show that BMS-189664 is efficacious at protecting mice from thrombin-induced lethality in vivo and at inhibiting arterial and venous thrombosis in monkeys. Based on these findings and additional pharmacokinetic and efficacy studies in other animal models, BMS-189664 was selected for further development.

## References and Notes

1. Das, J.; Kimball, S. D.; Reid, J. A.; Wang, T. C.; Lau, W. F.; Roberts, D. G. M.; Seiler, S. M.; Schumacher, W. A.; Ogletree, M. L. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 41.

2. Bis-BOC-guanidine **7** was synthesized from 4-aminomethylpiperidine (93% overall yield) by its reaction with benzaldehyde (1.1 equiv, toluene, Δ), followed by addition of bis-BOC-amidinopyrazole (1 equiv, rt) and subsequent hydrolysis (1 N aq $KHSO_4$, rt).

3. In vitro inhibition of thrombin catalytic activity using 10 μM substrate s-2238 (D-Phe-Pip-Arg-pNA) was measured at rt after 3 min incubation with inhibitor. For a description of the assay, see: Balasubramanian, N.; St. Laurent, D. R.; Federici, M. E.; Meanwell, N. A.; Wright, J. J.; Schumacher, W. A.; Seiler, S. M. *J. Med. Chem.* **1993**, *36*, 300. The $K_i$ values for competitive inhibitors can be determined from $IC_{50}$ values, see: Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharm.* **1973**, *22*, 3099. Each value represents an average of at least three determinations.

4. The model involved iv challenge with human α-thrombin (10–20 units/mouse) in anesthetized mice treated with the inhibitor either 10 min (iv) or 60 min (po) prior to thrombin challenge. $ID_{50}$ is the dose of the inhibitor required for 50% mice survival.

5. $K_i$ was determined by measuring enzyme activity at three different substrate (S) concentrations and six different inhibitor (I) concentrations, curve fitting the data to $V = V_{max}[S]/\{K_m(1 + [I]/K_i) + [S]\}$.

6. Malley, M. F.; Tabernero, L.; Chang, C. Y.; Ohringer, S.; Roberts, D. G. M.; Das, J.; Sack, J. S. *Protein Sci.* **1996**, *5*, 221.

7. Banner, D. W.; Hadvary, P. *J. Biol.Chem.* **1991**, *266*, 20085.

8. Bode, W.; Turk, D.; Karshikov, A. *Protein Sci.* **1992**, *1*, 426.

9. Schumacher, W. A.; Heran, C. L.; Goldenberg, H. J.; Harris, D. N.; Ogletree, M. L. *Am. J. Physiol.* **1989**, *256*, H726.