

# {4(S)-(Piperidin-1-ylcarbonyl)-4-[3-(trifluoromethyl)phenylsulfonamido]butyl}guanidinium chloride: a model of a graftable thrombin inhibitor

Catherine Michaux,<sup>a\*</sup> Claudio Salvagnini,<sup>b</sup> Bernadette Norberg,<sup>a</sup> Jacqueline Marchand-Brynaert<sup>b</sup> and Johan Wouters<sup>a</sup>

<sup>a</sup>Laboratoire de Chimie Biologique Structurale, University of Namur, 61 Rue de Bruxelles, B-5000 Namur, Belgium, and <sup>b</sup>Unité de Chimie Organique et Médicinale, Catholic University of Louvain, Batiment Lavoisier, Place Louis Pasteur 1, B-1348 Louvain-la-Neuve, Belgium

Correspondence e-mail: catherine.michaux@fundp.ac.be

Received 18 September 2006

Accepted 9 November 2006

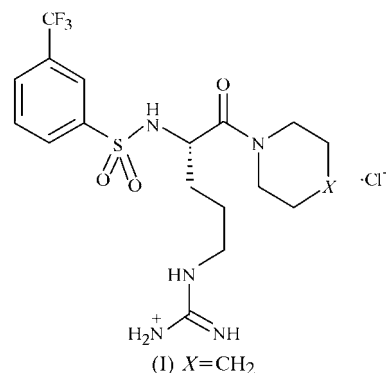
Online 22 November 2006

In the title compound,  $C_{18}H_{27}F_3N_5O_3S^+\cdot Cl^-$ , the guanidine group forms  $N-H\cdots Cl$  hydrogen bonds, with four  $N\cdots Cl$  distances in the range 3.164 (3)–3.337 (4) Å. In the crystal packing, the cations are further linked by  $N-H\cdots O$  and  $C-H\cdots O$  interactions. The structure is compared with that of argatroban complexed with thrombin and is the subject of docking studies in the active site of thrombin.

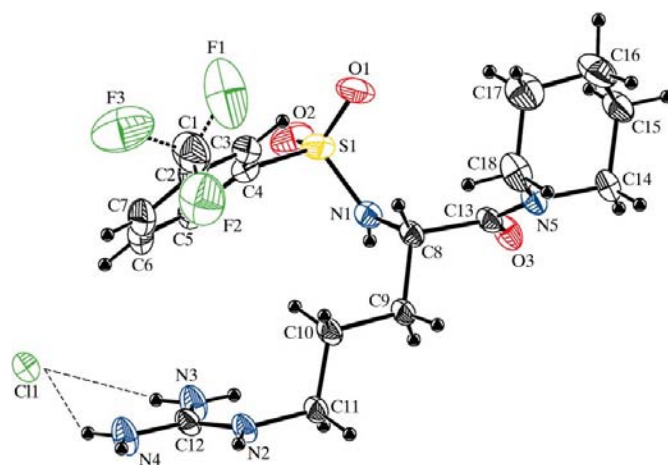
## Comment

Thrombin is a key enzyme in the blood coagulation system and represents a main target in medicinal chemistry (Weitz, 2003). Recently, we became interested in designing thrombin inhibitors for the biocompatibilization of polymer materials (Salvagnini *et al.*, 2005). Our strategy relies upon the covalent grafting of biologically active molecules, *via* a spacer arm, on the surface of polymer devices. In this way, we can selectively confer specific properties on the material surface, while the physicochemical and mechanical properties of the bulk remain unchanged (Marchand-Brynaert, 1999). Graftable thrombin inhibitors are derived from L-arginine and have a piperazinyl amide moiety bearing a spacer arm at position 4 (*X* in the scheme) for surface grafting. The replacement of the classically used piperidine with a piperazine ring, in order to use the atom at position 4 as an anchor for various spacer arms, had to be validated. Thus, a small library of thrombin inhibitors with different groups at position 4 was synthesized. Of these, the title compound with a  $CH_2$  substituent, (I) (with a piperidine group), is presented here. Four other compounds, *viz.* with  $X = O$ ,  $N-H$ ,  $N-Et$  and  $N-COCH_3$  substituents, will be published elsewhere (Salvagnini *et al.*, 2006).

The elucidation of the structure of (I) (Fig. 1) and its interactions in crystal packing can help to determine its position and orientation when docked in the thrombin active site. Bond lengths and angles are as expected, *e.g.* the  $S=O$  bond lengths in the sulfonamide group are similar to the expected value of 1.43 Å for  $C-SO_2-N$  systems (Allen, 2002). The N atom of the amide bond, involved in the piperidine ring, is  $sp^2$ , with bond angles close to  $120^\circ$  (total of  $358^\circ$ ) [ $C13-N5-C18 = 126.9$  (3)°,  $C13-N5-C14 = 118.3$  (3)° and  $C18-N5-C14 = 112.7$  (3)°].



In compound (I), the guanidine group interacts with the  $Cl^-$  anions (Table 1) *via* strong donor–weak acceptor  $N-H\cdots Cl^-$  interactions (Desiraju & Steiner, 1999) that can also be described as ‘chelated’ (Giacovazzo *et al.*, 1992). In the Cambridge Structural Database (CSD, Version 5.27; Allen, 2002), 36 entries have such interactions (*ISOSTAR*; Bruno *et al.*, 1997), but only a few possess the chelated geometry, where the  $Cl^-$  anion interacts with two  $N-H$  groups;  $N-H\cdots Cl$  bond distances are in the range 2.2–2.8 Å. In the crystal packing,  $N/C-H\cdots O$  interactions are present and link symmetry-related cations (Table 1). An intramolecular  $C-H\cdots \pi$ (arene) interaction ( $C10-H10B\cdots Cg2$ , where  $Cg2$  is the centroid of the  $C2-C7$  ring; Table 1) and weaker  $C-$



**Figure 1**

A view of (I), showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level. Thin dashed lines indicate the hydrogen bonds. Heavy dashed lines indicate the minor (42%) disordered  $CF_3$  group.

H···O contacts constrain the conformations of the guanidine, piperidine and trifluoromethylphenyl groups.

The amide and sulfonamide groups of (I) were superimposed on argatroban (a reference thrombin inhibitor) cocrystallized with human thrombin (Fig. 2*a*) (Banner & Hadvary, 1991). The piperidine group of each compound fits well, in contrast with the other groups. Indeed, the guanidine function is oriented so as to form either an ionic interaction with Asp189 in thrombin or an N—H···Cl interaction in the crystal packing. Moreover, the sulfonamide group of argatroban interacts with the backbone of Gly216, orienting the tetrahydroquinoline in a different way than the trifluorophenyl group of (I).

The structure of (I) was subjected to a docking study in the active site of human thrombin [Protein Data Bank (PDB; Berman *et al.*, 2000) code 1DWC (Banner & Hadvary, 1991)]

using *GOLD* (Jones *et al.*, 2001). The binding mode of (I) is similar to that of argatroban (r.m.s. deviation = 0.28 Å) (Fig. 2*b*). The guanidyl functional group, which binds the S-pocket and interacts with Asp189, is in an extended conformation and also interacts with the C=O bond of Gly219 and Ala190. The piperidine and trifluorophenyl groups occupy the P- and D-pockets, respectively. A weak O—H···F bond is observed between the CF<sub>3</sub> group and Tyr60A of the D-pocket (Desiraju & Steiner, 1999). This type of interaction is not common, but eight entries in the PDB involve a CF<sub>3</sub> group interacting with a tyrosine (*ISOSTAR*), with O···F distances in the range 2.9–3.4 Å.

In conclusion, the crystal structure of the title thrombin inhibitor, (I), was determined in order to highlight its structural properties. Moreover, it has proved to be a useful starting point for thrombin docking studies.

## Experimental

The synthesis of (I) has been described previously (Salvagnini *et al.*, 2005). Colourless crystals suitable for X-ray analysis were obtained by slow evaporation of an ethanol solution of (I).

### Crystal data

C<sub>18</sub>H<sub>27</sub>F<sub>3</sub>N<sub>5</sub>O<sub>3</sub>S<sup>+</sup>·Cl<sup>−</sup>  
 $M_r = 485.96$   
 Orthorhombic,  $P2_12_12_1$   
 $a = 8.3710$  (10) Å  
 $b = 9.3510$  (10) Å  
 $c = 29.554$  (3) Å  
 $V = 2313.4$  (4) Å<sup>3</sup>

$Z = 4$   
 $D_x = 1.395$  Mg m<sup>−3</sup>  
 Cu  $K\alpha$  radiation  
 $\mu = 2.79$  mm<sup>−1</sup>  
 $T = 293$  (2) K  
 Prism, colourless  
 0.45 × 0.21 × 0.04 mm

### Data collection

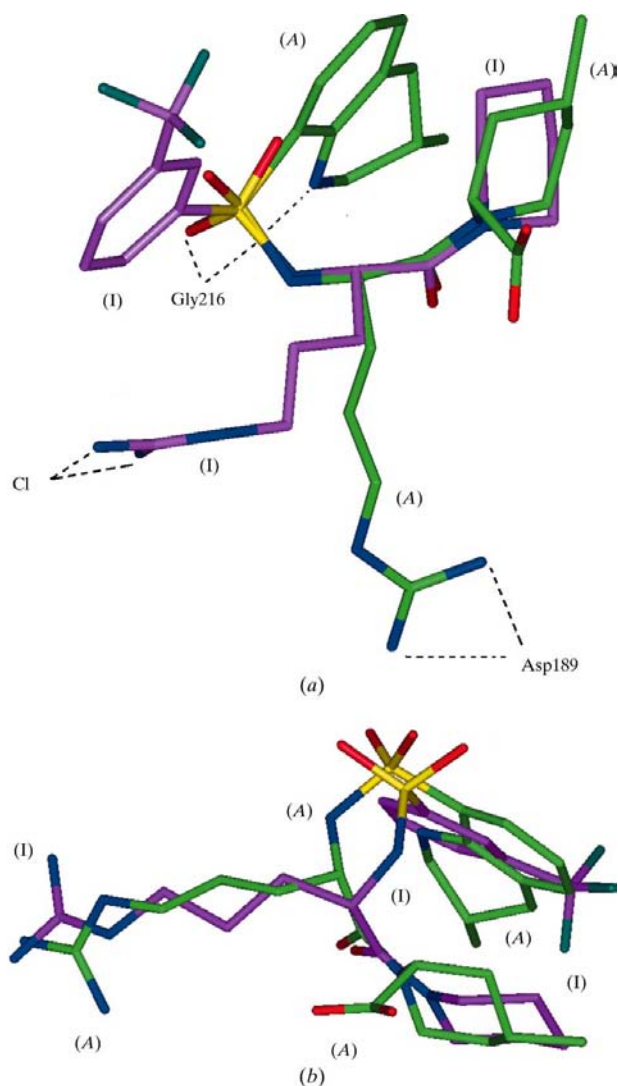
Enraf–Nonius CAD-4  
 diffractometer  
 $\theta/2\theta$  scans  
 Absorption correction: analytical  
 (de Meulenaer & Tompa, 1965)  
 $T_{\min} = 0.367$ ,  $T_{\max} = 0.897$   
 2740 measured reflections

2740 independent reflections  
 2579 reflections with  $I > 2\sigma(I)$   
 $\theta_{\max} = 75.0^\circ$   
 3 standard reflections  
 every 200 reflections  
 intensity decay: 2%

### Refinement

Refinement on  $F^2$   
 $R[F^2 > 2\sigma(F^2)] = 0.046$   
 $wR(F^2) = 0.132$   
 $S = 1.06$   
 2740 reflections  
 289 parameters  
 H-atom parameters constrained

$w = 1/[\sigma^2(F_o^2) + (0.0927P)^2 + 0.4728P]$   
 where  $P = (F_o^2 + 2F_c^2)/3$   
 $(\Delta/\sigma)_{\max} < 0.001$   
 $\Delta\rho_{\max} = 0.39$  e Å<sup>−3</sup>  
 $\Delta\rho_{\min} = -0.40$  e Å<sup>−3</sup>  
 Absolute structure: Flack (1983)  
 Flack parameter =  $-0.00$  (3)



**Figure 2**

(*a*) The conformation of the crystal structure of (I). (*b*) The conformation after docking in thrombin. These conformations are superimposed on the cocrystallized structure of argatroban (A) in the active site of human thrombin.

**Table 1**

Hydrogen-bond geometry (Å, °).

Cg2 is the centroid of the C2–C7 benzene ring.

$D-H\cdots A$	$D-H$	$H\cdots A$	$D\cdots A$	$D-H\cdots A$
N1–H1···Cl1 <sup>i</sup>	0.86	2.45	3.164 (3)	141
N2–H2···O3 <sup>iii</sup>	0.86	2.11	2.886 (4)	151
N3–H3A···Cl1 <sup>i</sup>	0.86	2.46	3.237 (3)	150
N3–H3B···Cl1	0.86	2.55	3.316 (3)	148
N4–H4A···Cl1	0.86	2.59	3.337 (4)	146
N4–H4B···O3 <sup>iii</sup>	0.86	2.11	2.873 (5)	148
C10–H10B···Cg2	0.97	2.93	3.688 (5)	136
C11–H11A···O3 <sup>iii</sup>	0.97	2.56	3.420 (4)	148

Symmetry codes: (i)  $-x + 1, y + \frac{1}{2}, -z + \frac{1}{2}$ ; (ii)  $x, y - 1, z$ ; (iii)  $-x, y - \frac{1}{2}, -z + \frac{1}{2}$ .

All H atoms were calculated and fixed in geometrically idealized positions using the *SHELXL97* defaults (at 293 K) (Sheldrick, 1997), with N—H = 0.86 Å and C—H = 0.93–0.98 Å, and with  $U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{C}, \text{N})$ . The CF<sub>3</sub> group is disordered over two rotational occupancies with site-occupancy factors of 0.58 and 0.42.

The stereochemistry of (I) has already been determined (Salvagnini *et al.*, 2005, 2006), and therefore no Friedel reflections were collected. The refined value of the Flack (1983) parameter of 0.00 (3) with  $R = 0.046$  confirmed the *S* configuration, whereas the inverted conformation (*R* configuration) gave a Flack parameter of 0.93 (2) for  $R = 0.059$ .

The docking studies were performed using the program *GOLD* (Jones *et al.*, 2001), which is a genetic algorithm for protein–ligand docking with full ligand and partial protein flexibility. The active site was defined as all protein atoms within 15 Å of any ligand atom in the experimental protein–ligand complex (PDB refcode 1DWC; Banner & Hadvary, 1991). A maximum of ten docking solutions were generated for each structure. The default software settings were used for the parameters controlling the genetic algorithm. The scoring function used to rank the dockings was GOLDScore and it is partly based on conformational and non-bonded contact information from the CSD.

Data collection: *CAD-4 MACH3* (Nonius, 2000); cell refinement: *CAD-4 MACH3*; data reduction: *HELENA* (Spek, 1997); program(s) used to solve structure: *SIR97* (Altomare *et al.*, 1999); program(s) used to refine structure: *SHELXL97* (Sheldrick, 1997); molecular graphics: *PLATON* (Spek, 2003); software used to prepare material for publication: *enCIFer* (Allen *et al.*, 2004).

CM thanks the FNRS for the award of a research fellowship. This work was supported by the FNRS and the Communauté Française de Belgique (Action de Recherche Concertée No. 99/04-240). JMB is a senior research associate of the FNRS.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: GG3050). Services for accessing these data are described at the back of the journal.

## References

- Allen, F. H. (2002). *Acta Cryst.* **B58**, 380–388.
- Allen, F. H., Johnson, O., Shields, G. P., Smith, B. R. & Towler, M. (2004). *J. Appl. Cryst.* **37**, 335–338.
- Altomare, A., Burla, M. C., Camalli, M., Cascarano, G. L., Giacovazzo, C., Guagliardi, A., Moliterni, A. G. G., Polidori, G. & Spagna, R. (1999). *J. Appl. Cryst.* **32**, 115–119.
- Banner, D. W. & Hadvary, P. (1991). *J. Biol. Chem.* **266**, 20085–20093.
- Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalow, I. N. & Bourne, P. E. (2000). *Nucleic Acids Res.* **28**, 235–242. (URL: <http://nar.oupjournals.org/cgi/content/full/28/1/235>.)
- Bruno, I. J., Cole, J. C., Lommerse, J. P., Rowland, R. S., Taylor, R. & Verdonk, M. L. J. (1997). *Comput. Aided Mol. Des.* **11**, 525–537.
- Desiraju, G. R. & Steiner, T. (1999). *The Weak Hydrogen Bond in Structural Chemistry and Biology*. New York: Oxford University Press Inc.
- Flack, H. D. (1983). *Acta Cryst.* **A39**, 876–881.
- Giacovazzo, C., Monaco, H. L., Viterbo, D., Scordari, F., Gilli, G., Zanotti, G. & Catti, M. (2002). *Fundamentals of Crystallography*, edited by C. Giacovazzo, pp. 1–825. New York: Oxford University Press Inc.
- Jones, G., Willett, P., Glen, R. C., Leach, A. R. & Taylor, R. (2001). *GOLD*. Version 1.2. Astex Technology, Cambridge, England.
- Marchand-Brynaert, J. (1999). *Surface Chemistry and Electrochemistry of Membranes*, edited by T. Sorensen, pp. 91–124. New York: Marcel Dekker Inc.
- Meulenaer, J. de & Tompa, H. (1965). *Acta Cryst.* **19**, 1014–1018.
- Nonius (2000). *CAD-4 MACH3*. Nonius BV and Delft Instruments, Delft, The Netherlands.
- Salvagnini, C., Gharbi, S., Boxus, T. & Marchand-Brynaert, J. (2006). *Eur. J. Med. Chem.* In the press.
- Salvagnini, C., Michaux, C., Remiche, J., Wouters, J., Charlier, P. & Marchand-Brynaert, J. (2005). *Org. Biomol. Chem.* **3**, 4209–4220.
- Sheldrick, G. M. (1997). *SHELXL97*. University of Göttingen, Germany.
- Spek, A. L. (1997). *HELENA*. Utrecht University, The Netherlands.
- Spek, A. L. (2003). *J. Appl. Cryst.* **36**, 7–13.
- Weitz, J. I. (2003). *Thromb. Res.* **109**, S17–S22.