Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2931–2934
Bioorganic & Medicinal Chemistry Letters

Sulfonamide-related conformational effects and their importance in structure-based design

Stefan Senger,* Chuen Chan, Máire A. Convery, Julia A. Hubbard, Gita P. Shah, Nigel S. Watson and Robert J. Young

GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, UK

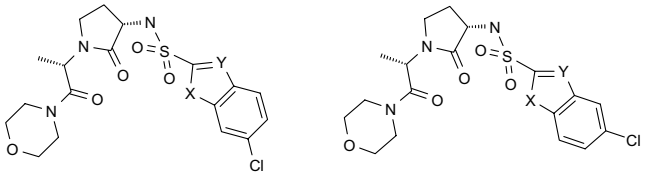
Received 19 January 2007; revised 12 February 2007; accepted 14 February 2007
Available online 16 February 2007

Dedicated to Prof. Dr. em. Günther Maier (Justus-Liebig-Universität Gießen, Germany) on the occasion of his 75th birthday.

Abstract—Structure-based design (SBD) is a challenging endeavour since even localised SAR can hardly ever be explained by the variation of just one dominating factor. Here, we present a rare example where structural information combined with ab initio calculations clearly indicate that the observed difference in biological activity is dominated by conformational effects. The learnings discussed are successfully put to the test and have the potential to be of general use as a qualitative guide in SBD efforts.

Over recent years an ever increasing amount of three-dimensional structural information[1] has become available for biological targets. This in turn stimulated a rapid growth in the importance of structure-based design (SBD)[2] in drug discovery. However, for SBD to be successful the wealth of structural information that is being generated must be translated into a sound understanding of the principles that govern molecular recognition. Since the interaction between ligand and biological target is a highly complex process, the underlying energetics are difficult to describe in a quantitative fashion. To simplify the calculation of binding energies, it is generally assumed that the different contributions can be calculated separately and that they are additive. Ajay and Murcko,[3] for example, separate the binding energy into contributions due to (1) the interaction of the reactants that form the (non-bonded) complex, (2) solvation/desolvation, (3) energy changes associated with changes in the 'motion' of the biological target and ligand when forming a complex (i.e., the loss of degrees of rotational and translational freedom) and (4) conformational changes of the ligand and the biological target during complexation.[4] Even when the binding of structurally closely related ligands to the same biological target is studied, it is rarely the case that the change in biological activity (i.e., binding energy) can be explained by the variation of one specific contribution alone, for example, conformational changes in the ligand. This makes it even more challenging to gain a better understanding of molecular recognition and, ultimately, to reliably calculate binding energies.

Recently, we have described our efforts which led to the discovery of a series of novel pyrrolidinone derivatives as potent factor Xa (FXa) inhibitors.[5] As part of this work we studied the structure–activity relationship (SAR) for a group of FXa inhibitors **1**–**4** containing chloro-substituted [5,6]-fused aromatics attached to a sulfonamide linker group.[6]



1a	X = S, Y = CH	**1b**
2a	X = S, Y = N	**2b**
3a	X = NH, Y = CH	**3b**
4a	X = O, Y = CH	**4b**

The crystallographically observed binding modes for inhibitors of this chemotype gave rise to the hypothesis that conformational effects[7] might play an important role in determining the relative activities of the

Keywords: Sulfonamide; Torsional scan; Conformational effects; Structure-based design; Thrombin.
* Corresponding author. Tel.: +44 (0) 1438 763959; fax: +44 (0) 1438 763352; e-mail: stefan.x.senger@gsk.com

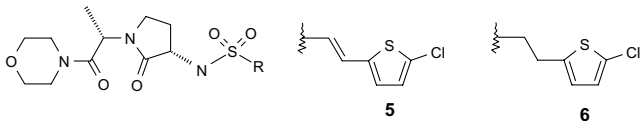
0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.02.034

6-chloro- (**1a**–**4a**) versus the 5-chloro-substituted (**1b**–**4b**) regioisomer in the four pairs **1**–**4**. Encouragingly, torsional scans of the sulfonamide S–C bonds in small model systems (at the B3LYP/6-31G* level of theory)[6] did indeed support the idea that the sulfonamide conformation might play an important role in determining the activity in this particular series of factor Xa inhibitors. Our findings prompted us to investigate further the question of the importance of sulfonamide-related conformational effects in the context of SBD. To the best of our knowledge, the relevance of the sulfonamide conformation in regard to ligand design has previously only been highlighted by Baldwin et al.[8]

The fact that the molecules in the pairs **1**–**4** are structurally extremely similar gave rise to the assumption that differences in activity could be explained by the variation of only one of the components contributing to the binding energy (i.e., ligand conformational energies). However, this is clearly an oversimplification. For example, we know from crystallographic studies that inhibitor **3b** forms a hydrogen bond with a backbone carbonyl (Gly218), whereas this hydrogen bond is not formed when **3a** binds to FXa.[6]

To further elucidate the importance of sulfonamide-related conformational effects, we have tried to identify (pairs of) ligands where it can be established, with a high degree of confidence, that the difference in binding energy is dominated by conformational effects of the ligand. Results from this studies are presented in this communication.

As we have recently described, our efforts to identify new antithrombotic agents led to the discovery of the potent and selective FXa inhibitor **5**.[5c] When probing the structural proximity of **5** we were very surprised to find that **6**[9] is approximately 20-fold more potent than **5** when tested against thrombin: K_i (**5**, thrombin) = 367 nM, K_i (**6**, thrombin) = 17 nM.[10]



5 **6**

In order to better understand this unexpected difference in thrombin activity, we determined the X-ray crystal structures of thrombin complexed with **5** and **6**, respectively.[11] However, as can be seen in Figure 1, both inhibitors bind to thrombin in an identical fashion. The RMSD for the two inhibitors bound to thrombin (and the protein atoms superposed) is 0.2 Å with a maximum distance between two equivalent atoms of only 0.4 Å (for one of the carbon atoms in the morpholino ring).

Although the X-ray structures of the two thrombin complexes did not provide us with the explanation we have been hoping for, they do indeed help to eliminate the possibility that a different interaction pattern between the ligands and the enzyme could be responsible for the observed difference in activity.

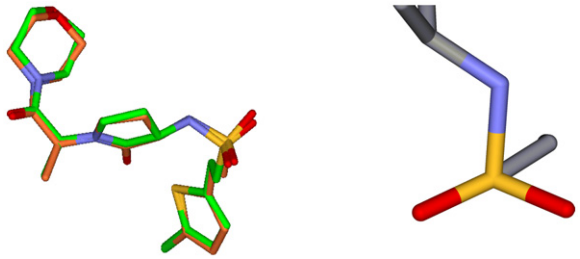


Figure 1. Comparison of the conformations of **5** (orange) and **6** (green) complexed to thrombin (left). On the right, a view along the S–C bond is shown for **5** bound to thrombin.

Similarly, the fact that **5** and **6** show an extremely high degree of structural similarity (i.e., the only difference being the bond order of a carbon–carbon bond) makes it unlikely that either a difference in energetic effects related to solvation/desolvation or a difference in the loss of degrees of rotational freedom on binding to the receptor can be the cause of the observed difference in activity. Considering what has been described by Ajay and Murcko[3] (see above), this only leaves conformational changes to explain the observed behaviour. Furthermore, since there is no significant difference in the conformation of the active site residues in the two thrombin complexes it appears most likely that the difference in activity can be solely explained through changes in the ligand conformation on binding to thrombin.

Based on the encouraging results from our previous study,[6] we decided to focus on the torsion (C=C)–(S=O) and (C–C)–(S=O) for **5** and **6**, respectively. Scans of the dihedral angles were performed in the simple model systems *N*-methylethenesulfonamide (**M1**) and *N*-methylethylsulfonamide (**M2**) with the Gaussian98[12] suite of programs at the B3LYP/6-31G* level of theory.[13] Stationary points located in this manner have subsequently been fully optimised and frequency calculations have been performed at the same level of theory. The results for **M1** are shown in Figure 2.

The energetically most preferred conformer of **M1** is the one where the carbon–carbon double bond is roughly in plane with the S=O moiety that is syn to the methyl group. The situation is different for the arrangement that is found when **5** is bound to thrombin. In the conformation found in the complex (cf. green circle in Fig. 2) the vinyl group is rotated by approximately 185° when compared with the energetically most preferred conformer which makes this arrangement approximately 3 kcal/mol higher in energy.

The results of the torsional scan of model system **M2** show that the situation is totally different for **6** (cf. Fig. 3). The dihedral angle for (C–C)–(S=O) found when **6** is complexed with thrombin is identical to one of the three low energy conformations and hence there is no conformational energy penalty (for the (C–C)–(S=O) torsion) when **6** binds to thrombin. These findings are in good agreement with the experimental obser-

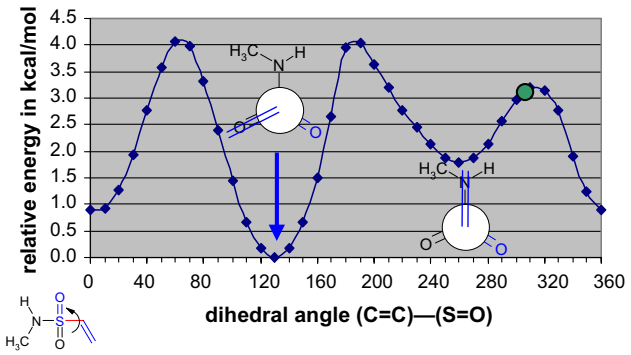


Figure 2. Relaxed scan of the dihedral angle (C=C)–(S=O) (in 10° increments; the oxygen atom is the one anti to the methyl group) in *N*-methylethenesulfonamide (**M1**). The green circle is placed at the dihedral angle found in **5** bound to thrombin (cf. Fig. 1).[11]

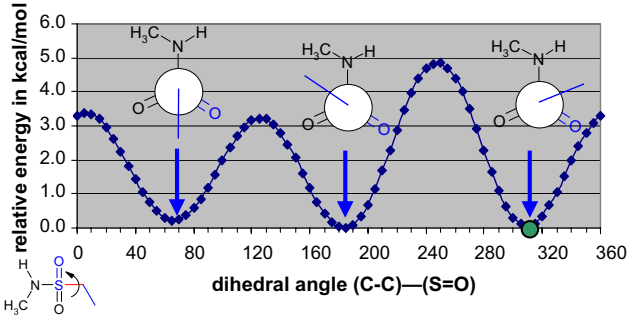


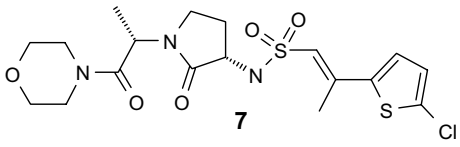
Figure 3. Rigid scan of the dihedral angle (C–C)–(S=O) (in 5° increments; the oxygen atom is the one anti to the methyl group) in *N*-methylethanesulfonamide (**M2**). No geometry optimisations (for the non-scanned coordinates) were performed at the sampling points. The green circle is placed at the dihedral angle found in **6** bound to thrombin.[11]

vation that (all else being equal) **6** should be a more potent thrombin inhibitor than **5**.

In order to establish the relevance of the torsion between the thiophene and the adjacent carbon–carbon double or single bond in **5** or **6**, respectively, we have also performed torsional scans for 2-ethenethiophene and 2-ethylthiophene.[16] The trend observed for this torsion is the same as what has been discussed above, that is, the conformational energy penalty for **5** is higher than that for **6**. However, when comparing the contribution of a specific torsion to the relative conformational energy penalty (**5** vs **6**) the effect is more distinct for (C=C)–(S=O)/(C–C)–(S=O) (3 kcal/mol) than it is for torsion between the thiophene ring and the adjacent C=C/C–C (0.9 kcal/mol).

In summary, the results discussed above support the initial hypothesis that the observed difference of thrombin activity for **5** and **6** can be explained by conformational effects. Analyses of torsional profiles of the two torsions adjacent to the carbon–carbon bond in the model systems **M1** and **M2** lead to the conclusion that the conformational energy penalty for binding to thrombin is smaller for **6** than it is for **5**. Furthermore, our results indicate that the torsion adjacent to the sulfonamide sulfur atom has a more distinct effect than the torsion next to the thiophene ring.

In an attempt to test the learnings from our studies,[6] we tried to predict how the thrombin activity of **5** could be increased through a structural modification other than changing the bond order of the carbon–carbon double bond. To achieve this, we would have to stabilise the conformation of the dihedral angle (C=C)–(S=O) that is found in the thrombin complex (see Fig. 1 and green circle in Fig. 2). One way of doing this is to attach a methyl group to the carbon atom adjacent to the thiophene ring, resulting in **7**.



7

A torsional scan performed for the model system *N*-methyl-2-propenesulfonamide (**M3**) (cf. Fig. 4) confirms that the relative energy of the conformation found in the thrombin complex is only approximately 1.3 kcal/mol, that is, significantly less than the 3 kcal/mol for **M1**.

Consequently, we can expect **7** to be a more potent thrombin inhibitor than **5** (whilst assuming that **7** binds to thrombin in the similar way as **5** and **6**). Very encouragingly, this was indeed observed. When tested against thrombin, **7** was reported to have a K_i of 2 nM.[10]

Based on the calculated relative conformational energies alone it might be surprising to find **7** to be a more potent thrombin inhibitor than **6**. However, it must be taken into account that **7** contains an additional methyl group which can favourably interact with residues in the active site of thrombin as well as slightly perturb the atomic positions of the ligand in the active site (when compared to **5** bound to thrombin). Our X-ray crystallographic studies[11] confirm that the latter is indeed the case. The RMSD for **7** relative to **5** when bound to thrombin (and the protein atoms are superposed) is 0.6 Å with a maximum distance between two equivalent atoms of 1.0 Å (for one of the carbon atoms in the pyrrolidinone ring).

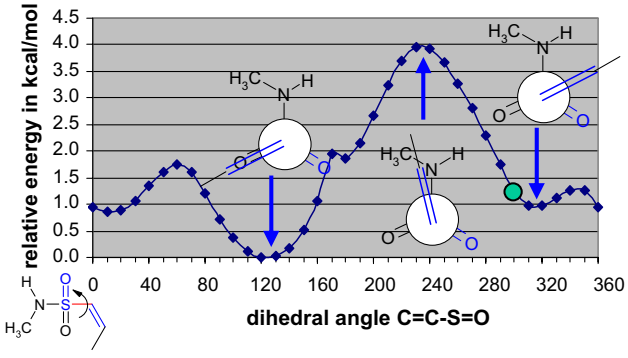


Figure 4. Relaxed scan of the dihedral angle (C=C)–(S=O) (in 10° increments) in *N*-methyl-2-propene-1-sulfonamide (**M3**). The green circle is placed at the dihedral angle found in **7** bound to thrombin.[11]

This example nicely illustrates how the findings from this study can be used to guide SBD efforts in a qualitative fashion.

The study presented here strongly supports our previous findings[6] suggesting that sulfonamide-related conformational effects can play a significant role in molecular recognition. Future studies will show how important a careful consideration of these effects is in the context of structure-based design.

Acknowledgment

We gratefully acknowledge the contributions of the members of the factor Xa programme team.

References and notes

1. See, for example: http://www.pdb.org.
2. (a) *Structure-Based Drug Design*; Veerapandian, P., Ed.; Marcel Dekker: New York, 1997; (b) *Structure-Based Ligand Design: Methods and Principles in Medicinal Chemistry*; Gubernator, K., Böhm, H.-J., Eds.; Wiley-VCH: Weinheim, 1998; Vol. 6, (c) Anderson, A. C. *Chem. Biol.* **2003**, *10*, 787; (d) *Structure-Based Drug Discovery—An Overview*; Hubbard, R. E., Ed.; RSC: Cambridge, 2006; (e) Tintelnot-Blomley, M.; Lewis, R. A. *IDrugs* **2006**, *9*, 114.
3. Ajay; Murcko, M. A. *J. Med. Chem.* **1995**, *38*, 4953.
4. Raha, K.; Merz, K. M., Jr. *Ann. Rep. Comp. Chem.* **2005**, *1*, 113.
5. (a) Watson, N. S.; Brown, D.; Campbell, M.; Chan, C.; Chaudry, L.; Convery, M. A.; Fenwick, R.; Hamblin, J. N.; Haslam, C.; Kelly, H. A.; King, N. P.; Kurtis, C. L.; Leach, A. R.; Manchee, G. R.; Mason, A. M.; Mitchell, C.; Patel, C.; Patel, V. K.; Senger, S.; Shah, G. P.; Weston, H. E.; Whitworth, C.; Young, R. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3784; (b) Young, R. J.; Campbell, M.; Borthwick, A. D.; Brown, D.; Burns-Kurtis, C. L.; Chan, C.; Convery, M. A.; Crowe, M. C.; Dayal, S.; Diallo, H.; Kelly, H. A.; King, N. P.; Kleanthous, S.; Mason, A. M.; Mordaunt, J. E.; Patel, C.; Pateman, A. J.; Senger, S.; Shah, G. P.; Smith, P. W.; Watson, N. S.; Weston, H. E.; Zhou, P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5953; (c) Chan, C.; Borthwick, A. D.; Brown, D.; Campbell, M.; Chaudry, L.; Chung, C.; Convery, M. A.; Hamblin, J. N.; Johnstone, L.; Kelly, H. A.; Kleanthous, S.; Kurtis, C. L.; Patikis, A.; Patel, C.; Pateman, A. J.; Senger, S.; Shah, G. P.; Toomey, J. R.; Watson, N. S.; Weston, H. E.; Whitworth, C.; Young, R. J.; Zhou, P. *J. Med. Chem.*, **2006**, in press.
6. Senger, S.; Convery, M. A.; Chan, C.; Watson, N. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5731.
7. Nicklaus, M. C.; Wang, S.; Driscoll, J. S.; Milne, G. W. A. *Bioorg. Med. Chem.* **1995**, *3*, 411.
8. Baldwin, J. J.; Ponticello, G. S.; Anderson, P. S.; Christy, M. E.; Murcko, M. A.; Randall, W. C.; Schwam, H.; Sugrue, M. F.; Springer, J. P.; Gautheron, P.; Grove, J.; Mallorga, P.; Viader, M.-P.; McKeever, B. M.; Navia, M. A. *J. Med. Chem.* **1989**, *32*, 2510.
9. Borthwick, A. D.; Chan, C.; Kelly, H. A.; Peace, S.; Senger, S.; Shah, G. P.; Smith, S. A.; Smith, S.; Watson, N. S.; West, R. I.; Young, R. J. WO 04/052851.
10. Standard deviations: compound **5**, 22 nM; compound **6**, 3 nM; compound **7**, 1 nM. Standard deviations are derived from at least $n \geqslant 2$. Experimental details for the fluorogenic thrombin assay can be found in Ref. 9.
11. The crystal structures of the thrombin complexes were obtained using essentially the method outlined in: Jhoti, H.; Cleasby, A.; Reid, S.; Thomas, P. J.; Weir, M.; Wonacott, A. *Biochemistry* **1999**, *38*, 7969. There was clear density for the ligands in initial difference Fourier maps. The structures for **5**, **6** and **7** were refined at 2.5, 2.2 and 1.7 Å, respectively, to a final $R_{\text{factor}}/R_{\text{free}}$ of 0.185/0.231 (**5**), 0.163/0.196 (**6**), 0.183/0.210 (**7**). Co-ordinates are deposited in the protein data bank with codes 2jh5, 2jh6, 2hj0.
12. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzeski, V. G.; Montgomery Jr., J. A.; Stratman, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G.A.; Ayala, P.Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; J. A. Pople Gaussian 98, Revision A.7, Gaussian, Inc.: Pittsburgh PA, 1998.
13. In the model system the nitrogen configuration is such that the nitrogen lone pair is synperiplanar to the C–S bond. Calculations at the B3LYP/6-31G* level of theory show that this arrangement is energetically preferred. This finding is in accordance with what has previously been reported at HF/6-31G* level of theory[14] for model system **M1**. Bindal et al.[15] have studied *N*-methylmethanesulfonamide and argue that the synperiplanar arrangement is more stable because of the avoidance of electron–electron repulsion between the lone pair on the nitrogen and the oxygens of the S=O bonds.
14. Gennari, C.; Salom, B.; Potenza, D.; Longari, C.; Fioravanzo, E.; Carugo, O.; Sardone, N. *Chem. Eur. J.* **1996**, *2*, 644.
15. Bindal, R. D.; Golab, J. T.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1990**, *112*, 7861.
16. For 2-ethenethiophene (the model system for **5**) the conformation found in the thrombin complex is approximately 1.6 kcal/mol higher in energy than the conformer with the lowest energy. For 2-ethylthiophene (the model system for **6**) the bound conformation is 0.7 kcal/mol higher in energy.