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Arylsulfonamides: A study of the relationship between activity and conformational preferences for a series of factor Xa inhibitors

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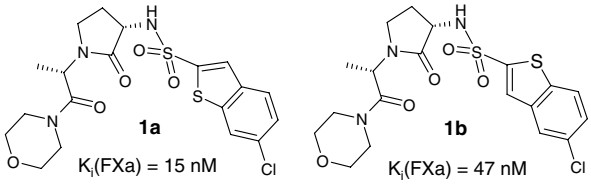
Abstract—Torsional scans of sulfonamide S–C bonds in small model systems of a series of arylsulfonamide factor Xa inhibitors were performed in order to investigate if conformational effects can help to rationalise the observed SAR. Computational results were in good agreement with the experimental data indicating that the sulfonamide conformation plays an important role in determining the activity in this particular series of factor Xa inhibitors.

Factor Xa is a trypsin-like serine proteinase that is located at the junction between the extrinsic and intrinsic coagulation pathways. It is the sole enzyme within the blood coagulation cascade responsible for activation of thrombin, leading to blood clot formation. Because of this important role in blood coagulation, factor Xa has emerged as an attractive target for development of new antithrombotic agents.[1]

Our own efforts have recently led to the discovery of a series of novel pyrrolidinone derivatives as potent factor Xa (FXa) inhibitors.[2] As part of this work we have synthesised inhibitors containing chloro-substituted [5,6]-fused aromatics attached to a sulfonamide linker group, for example **1a** and **1b**.



The X-ray crystal structures of factor Xa complexed with **1a** and **1b**, respectively, confirmed that the chloro-substituted fused aromatic rings bind in the S1 pocket of factor Xa with the chlorine in direct proximity to Ala190, Val213, Ile227 and Tyr228.[3] This positioning had been found previously for several other factor Xa–ligand complexes.[4] In accordance with the fact that **1a** and **1b** are closely structurally related, it is observed that they bind to the enzyme in a very similar fashion. The critical difference is the positioning of the heteroatom, that is, the sulfur, of the [5,6]-fused aromatic system. This is illustrated in Figure 1.

In the case of **1a**, one of the sulfur–oxygen bonds of the sulfonamide group is almost in plane with the carbon–carbon bond of the five-membered aromatic ring. On the other hand, in **1b** the sulfur–carbon bond of the five-membered aromatic ring is roughly in plane with the S–O bond. The question arose as to whether one of these conformations is energetically preferred and, furthermore, how the relative conformational energies relate to the perceived difference in activity between **1a** and **1b**. Even though it seemed unlikely that the apparent difference in activity can solely be rationalised by differences in the relative conformational energies of the inhibitors (i.e., the change in conformational energy when the inhibitor forms a complex with factor Xa) it was felt that a conformational study should be undertaken to investigate the importance of this factor. This seemed particularly useful since the Cambridge Crystallographic Database (CSD) contains only a very small number of entries with a five-membered aromatic ring directly attached to a sulfonamide sulfur atom.

The relaxed scan (in 10° increments) of the dihedral angles O–S–C–S (whereby the oxygen atom is the one

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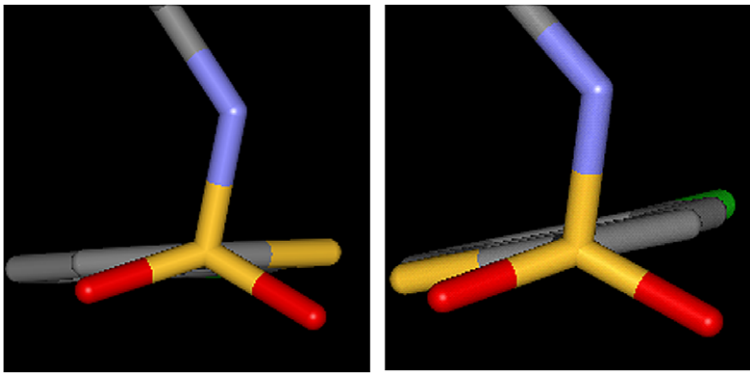

Figure 1. Comparison of the dihedral angle O–S–C–S (whereby the oxygen atom is the one syn to the C–N bond) in **1a** (left, 196°) and **1b** (right, 8°) bound to factor Xa.

syn to the C–N bond) in the simple model system *N*-methyl-2-thiophenesulfonamide (**M1**) (cf. Fig. 2) was performed with the Gaussian98 suite of programs[5] at the B3LYP/6-31G* level of theory. 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. There is a good agreement between the experimentally observed dihedral angle O–S–C–S of 196° in the bound conformation of **1a** (cf. Fig. 1) and the related dihedral angle of 185° in conformer C_2 of **M1**. The same is true for the experimentally observed dihedral angle O–S–C–S of 8° in the bound conformation of **1b** (cf. Fig. 1) and the related dihedral angle of 14° in conformer C_1 of **M1**.

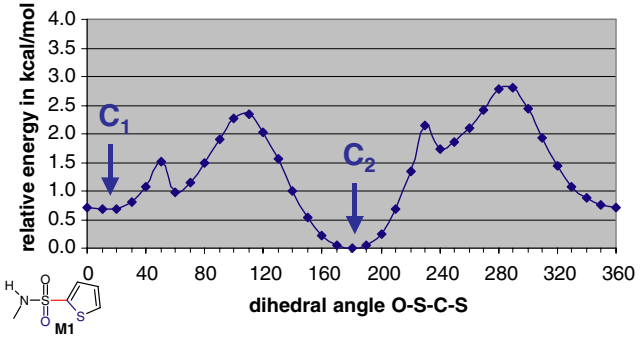

Figure 2. Relaxed scan of the dihedral angle O–S–C–S (in 10° increments, the oxygen atom is the one syn to the methyl group) in *N*-methyl-2-thiophenesulfonamide (**M1**).

The calculated energy difference for conformers C_1 and C_2 is 0.6 kcal/mol which corresponds to an expected difference in K_i of a factor of 2.8. This is in close agreement with the experimentally observed K_i ratio of 2.9. These encouraging results are in good agreement with the hypothesis that relative conformational energies might be an important factor in rationalising the SAR of series of inhibitors containing five-membered heteroaromatic rings attached to a sulfonamide linker. To further test this hypothesis, the conformational preferences of three additional pairs of inhibitors (cf. Fig. 3) have also been studied (using the same computational procedure). Based on the earlier example, it is assumed that the chlorine atom for all the compounds shown in Figure 3 is likely to be located in the small subpocket (formed by Ala190, Val213, Ile227 and Tyr 228) at the bottom of the S1 pocket.

Consequently, as has been already discussed for **1a** and **1b**, the critical difference will be the positioning of the heteroatom(s) of the [5,6]-fused aromatic system relative to the sulfur–oxygen bonds of the sulfonamide linker.

This is illustrated in Figure 4. In two of the three additional pairs the five-membered aromatic ring contains a polar heteroatom (**M2** and **M4**). In case of the third additional example a hydrogen-bond donor feature is present (**M3**). Since positioning these atoms differently in the S1 pocket of factor Xa will have a much more significant effect than the positioning of the sulfur atom, the results of the conformational scans had to be interpreted carefully. The results of the relaxed scans for the model systems **M2**–**M4** are shown in Figure 5.

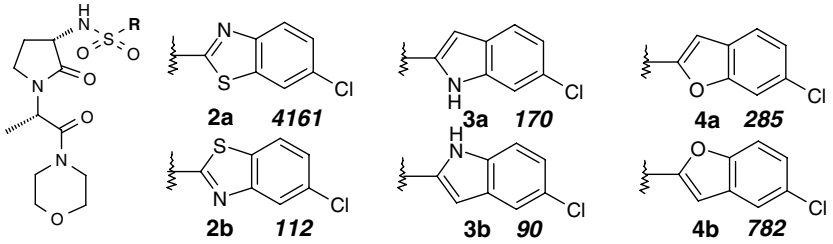

Figure 3. Factor Xa activities (K_i in nM, shown in bold italics) of six members of the pyrrolidone series.

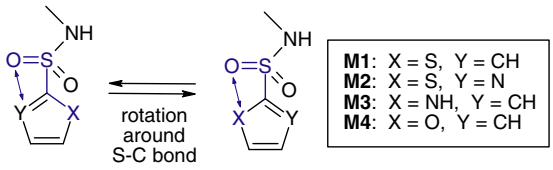

Figure 4. Simplified model systems **M1**–**M4** for **1a**, **1b** and the six factor Xa inhibitors shown in Figure 3. **M1**–**M4** were used to perform torsional scans around the S–C bonds. For the dihedral angle O–S–C–X the oxygen atom which is syn to the C–N bond was chosen.

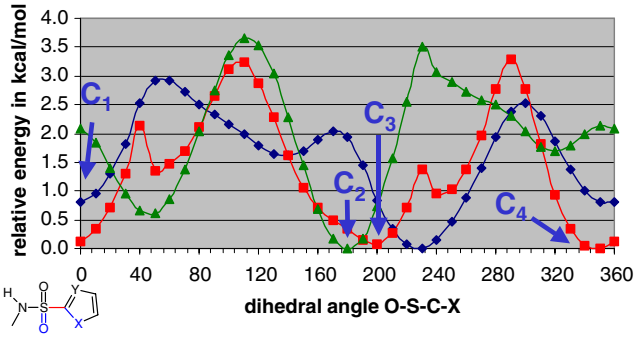

Figure 5. Relaxed scans of the dihedral angles O–S–C–X (in 10° increments, the oxygen atom is the one syn to the methyl group) in **M2** (blue), **M3** (red) and **M4** (green).

As outlined above, it seems reasonable to assume that all the factor Xa inhibitors **1**–**4** have very similar binding modes. Therefore, we anticipate the dihedral angles O–S–C–X in **2b** (X = S), **3b** (X = NH) and **4b** (X = O) to be in the proximity of 0° (i.e., one of the O–S bonds being roughly co-planar with the C–X bond as observed in the X-ray crystal structure of **1b** complexed with factor Xa,[3] cf. Fig. 1). By analogy, we expect the dihedral angle O–S–C–X in **2a** (X = S), **3a** (X = NH) and **4a** (X = O) to be in the proximity of 180° (cf. **1a** in Fig. 1). Table 1 lists the conformers of the model systems which have dihedral angles O–S–C–X of 0 ± 20° and 180 ± 20°, respectively, together with their calculated relative energies and the activities of the related factor Xa inhibitors.

In the model system **M2** there is no conformer with a dihedral angle O–S–C–S of approximately 180° (cf. Fig. 5). However, **M2** is in a minimum energy conformation (conf. C_1) if the dihedral angle O–S–C–S is 4°, corresponding to the anticipated bound conformation for **2b** (cf. Table 1). Consequently, the prediction would be that (as far as conformational preferences are concerned) **2b** is more active than **2a**. This is in good agreement with the experimental observations.

For the model system **M3** conformers with dihedral angles O–S–C–N of approx. 0° (conf. C_4) and 180° (conf. C_3), respectively, can be found (cf. Fig. 5). The calculated relative energies of conformers C_3 and C_4 give rise to the assumption that (as far as conformational aspects are concerned) **3a** and **3b** should be equipotent (cf. Table 1). As can be seen in Table 1, **3b** is reported to be approximately 2 times more potent than **3a**. This is in reasonable agreement with the prediction. The small discrepancy between predicted and observed relative activity might be partially due to the potential of the indole N–H in **3b** to form a hydrogen bond with the backbone carbonyl group of Gly218.[6] That the indole N–H in **3b** does indeed form a hydrogen bond with Gly218 was subsequently confirmed by X-ray crystallography.[3]

For model system **M4** a conformer can be found close to the dihedral angle O–S–C–O of approx. 180° (conf. C_2). No conformer is located in the proximity of the dihedral angle O–S–C–O of approximately 0° (cf. Table 1). This leads to the conclusion that (as far as conformational aspects are concerned) **4a** should be more active than **4b**. This prediction ties in nicely with the experimental results.

Based on the assumption that the SAR discussed here is dominated by conformational effects and with the help of the results obtained from the relaxed scans of the dihedral angles O–S–C–X (see Figs. 2 and 5) in the model systems **M1**–**M4** it was predicted which of the inhibitors in the four isomeric pairs **1**–**4** is expected to be more active against factor Xa. As can be seen in Table 1, our predictions have been correct in 3 out of the 4 cases in so far as the inhibitor that is more active against factor Xa

Table 1. Calculated relative energies E_{rel} (zero-point energy corrected, in kcal/mol), dihedral angles Θ (O–S–C–X, where the oxygen atom is the one syn to the methyl group), and activities (K_i in nM) of the corresponding factor Xa inhibitors fXaI for conformers with dihedral angles O–S–C–X in the range of 0 ± 20° and 180 ± 20°, respectively, of the fully optimised model systems **M1**–**M4**

Model[a]	X[a]	Y[a]	Conf.[b]	Θ[c]	E_{rel}[c]	fXaI	K_i[d]
M1	S	CH	C_1	14°	0.6	**1b**	47
M1	S	CH	C_2	185°	0.0	**1a**	15
M2	S	N	C_1	−4°	0.6	**2b**	112
M2	S	N	—	—	—	**2a**	4161
M3	NH	CH	C_4	−13°	0.0	**3b**	90
M3	NH	CH	C_3	198°	0.0	**3a**	170
M4	O	CH	—	—	—	**4b**	782
M4	O	CH	C_2	180°	0.0	**4a**	285

[a] cf. Figure 4.
[b] See relaxed scan plots. **M1**: Figure 2, **M2**–**M4**: Figure 5. If no conformer exists with a dihedral angle in the range of 0 ± 20° or 180 ± 20°, respectively, this is marked with '—'.
[c] Values refer to fully optimised structures.
[d] Standard deviations: **1b**, 3; **1a**, 5; **2b**, 7; **2a**, 1711; **3b**, 21; **3a**, 11; **4b**, 208; **4a**, 14. Standard deviations are derived from at least $n \geqslant 2$.

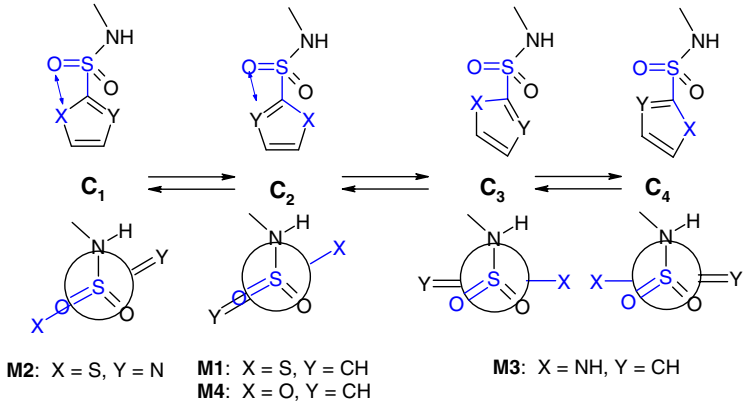

Figure 6. Preferred conformations C_1–C_4 of the model systems **M1**–**M4** (cf. Fig. 4) used in this study.

has been associated with a low energy conformer in the respective model system. In the case of **3a/3b** it was predicted that both inhibitors should be equipotent whereas the experimental results show that **3b** is 2-fold more potent. However, in this particular case there are indications that this might be caused by an attractive interaction between the indole NH of **3b** and the backbone carbonyl of Gly218 in the S1 pocket. In terms of the study presented here, we see this as a very encouraging outcome. Nonetheless, it must be stressed that the relative conformational energies are only a small contribution to the overall binding energy and that a trend between relative conformational energies and binding activities can only be observed due to the fact that we have restricted ourselves to compare isomeric molecules where other contributions to the binding energy are at a comparable level. The indole example **3a/3b** nicely illustrates that even in the case of isomeric molecules it is vital to consider carefully, on a case-by-case basis, other energy contributions (e.g. from H-bond donor interactions) that might have to be taken into account. Notwithstanding the above, we see value in performing this type of careful analysis since it may lead to qualitative insights which can be used to guide ligand design.

The results of the relaxed torsional scans performed for the model systems **M1**–**M4** indicate that there is a strong preference for one of the sulfonamide O–S bonds to be co-planar with the aromatic rings attached to the sulfur atom. Conformation C_2 (see Fig. 6) is preferred if a hydrogen atom is attached to Y and no hydrogen atom is connected to X (as in **M1** and **M4**). If X as well as Y form a bond to a hydrogen atom (as in **M3**) a conformation seems to be preferred in which both groups are close to planarity with the S–O bonds but none of them actually being co-planar (C_3 and C_4 in Fig. 6). If on the other hand, X is a sulfur atom and Y is not connected to an hydrogen atom (as in **M2**) C_1 seems to be the preferred conformation.

If in the conformation of a free inhibitor such a co-planar arrangement between the aromatic ring and an S–O bond is favoured but this arrangement is not possible when bound to the target an energy penalty for this loss is to be expected. The same should be the case when such an arrangement is absent in the free state but is observed when bound to the target. The relaxed scans presented here should prove useful in the structure-based design of sulfonamide-containing ligands.

In this study, we performed relaxed torsional scans of the S–C bond for the four small model systems **M1**–**M4** at the B3LYP/6-31G* level of theory. The results have been used to investigate if conformational effects can help to rationalise the observed SAR of a series of factor Xa inhibitors. Encouragingly, the computational results were in good agreement with the experimental data. We take this as an indication that the sulfonamide conformation plays an important role in determining the activity in this particular series of factor Xa inhibitors. To the best of our knowledge, the importance of the sulfonamide conformation in the context of inhibitor design has previously only been highlighted by J.J. Baldwin et al.[7] for the hCAII inhibition by thienothiopyran-2-sulfonamides.

Future structure-based design (SBD) efforts and structural studies of sulfonamide-containing inhibitor complexes will have to show how important a careful consideration of sulfonamide-related conformational effects is in the context of SBD.

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3. The complexes showed clear density for the ligand in F_o–F_c difference maps. The structures for **1a**, **1b** and **3b** were refined at 2.0, 2.1, and 1.9 Å, respectively, to a final R_{factor}/R_{free} of 0.178/0.222 (**1a**), 0.211/0.302 (**1b**) and 0.194/0.236 (**3b**), using procedures described in Refs. 2b,2c. Coordinates are deposited in the protein data bank with codes 2j34, 2j38 and 2j2u.
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