X-ray structures of elastase complexes to elucidate the mechanism of inhibition

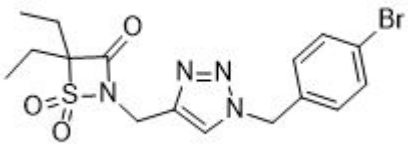
V. Almeida[1], L. Carvalho[1], M. Alves[1], S. Lucas[2], R. Moreira[2], M. Archer[1]

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, ITQB NOVA, Oeiras, Portugal, [2]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal, **archer@itqb.unl.pt**

Human neutrophil elastase (HNE) is a serine enzyme, which is one of the most destructive proteolytic enzymes, being able to catalyse the hydrolysis of connective tissue components. Its malfunction has been implicated in the development of diseases such as emphysema, cystic fibrosis and rheumatoid arthritis. The inhibition of proteolytic enzymes is a common goal for designing novel therapeutic agents, so many efforts have been made to find small molecule inhibitors of HNE [1].

Most structural and inhibition studies have been conducted with the related, but more readily available, porcine pancreatic elastase (PPE). The majority of elastase inhibitors often act by acylating the nucleophilic hydroxy group of serine-195 in the active site of the enzyme [2]. Sulfonylation of serine proteases represents an interesting strategy of inhibition as an alternative to the mechanism-based acylation process [3,4], but still not largely explored. Nucleophilic attack on 3-oxo-β-sultams compounds could involve either acylation with substitution at the carbonyl center and formation of the sulfonamide or sulfonylation resulting from substitution at the sulfonyl center and formation of the amide.

We have determined the 3D structures of PPE in complex with various inhibitors (see figure below) at atomic resolution (~1.4 Å) in order to clarify their mechanism of action.



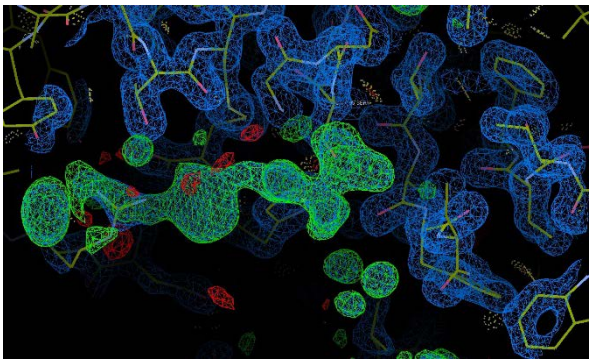


Figure: Co-crystallized inhibitor with the respective electron density map at the active site (Fo-Fc is represented in green mesh).

References

[1] - P.R. Bernstein, P.D. Edwards, J.C. Williams. Inhibitors of human leukocyte elastase. Prog. Med. Chem. **31**, 59 (1994).

[2] - J. Mulchande, L. Martins, R. Moreira, M. Archer, T.F. Oliveira, J. Iley, The efficiency of C-4 substituents in activating the β-lactam scaffold towards serine proteases and hydroxide ion. Org. Biomol. Chem. **5**, 2617(2007).

[3] - M. Beardsell, P.S. Hinchliffe, J.M. Wood, R.P. Wilmouth, C.J. Schofieldb, M.I. Page, β-Sultams - A novel class of serine protease inhibitors. Chem. Commun. **2001**, 497 (2001).

[4] - Y. Wing, T.N. Ahmed, L.P. Harding, K. Hemming, A.P. Laws, M.I. Page, Acylation versus Sulfonylation in the Inhibition of Elastase by 3-Oxo-β-Sultams. J. Am. Chem. Soc. **127**, 8946 (2005).