synthetic peptides with the general sequence D-Phe-Pro-D-Arg-X-$CONH_2$. These compounds are non-cleavable, reversible thrombin inhibitors and potent anticoagulants, as demonstrated by isothermal titration calorimetry and structure-activity relationship studies [2]. In order to characterize their molecular interactions with human thrombin, we solved the three-dimensional structure of the enzyme in complex with peptides with Ile (p3), Cys (p4) or D-Thr (p6) at the P1' position [3].

All the inhibitors bind in a substrate-like manner to the active site of thrombin. Although the contacts established by the P1 D-Arg with the enzyme are similar to those observed for the L-isomer in other substrates and inhibitors, it also allows for a deeper insertion of the P2-P3 segment into the respective selectivity pockets, and gives the cleavable bond an unfavorable geometry for nucleophilic attack by thrombin's Ser195 side chain. The latter occupies its canonical position in the thrombin-p3 complex, but in the p4 and p6 complexes it is rotated away from the catalytic histidine and hydrogen-bonded to the carbonyl oxygen of the P1' residue.

The side chain of Lys60F is thought to limit the size of thrombin's S1' pocket contributing to the frequent occurrence of small residues at this position in natural protein substrates. In the thrombin-p6 and -p4 complexes, the side chain of Lys60F is found in an extended conformation similar to that observed in the unliganded enzyme. However, in the thrombin-p3 complex, accommodation of the P1' isoleucine side chain implies the displacement of Lys60F, which is found in a different conformation similar to that observed in complexes of thrombin with bivalent inhibitors with a bulky residue (Nle or Thi) at the P1' position.

In brief, peptides with the general formula D-Phe-Pro-D-Arg-X-$CONH_2$ act as anti-coagulants by binding to thrombin in a substrate-like orientation. However, the presence of a D-Arg residue at position P1 impairs cleavage by the proteinase, preserves the interactions with the non-primed specificity subsites S1 to S3, and allows for the establishment of additional interactions by the residue in position P1', contributing for the observed high affinity of the peptides towards thrombin.

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Keywords: anticoagulant, peptide, thrombin

MS16.P69

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X-Ray crystallographic studies of rationally designed dihydroorotate dehydrogenase inhibitors

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The *de novo* pyrimidine biosynthesis pathway represents an attractive and validated drug target in a variety of organisms, including the malaria parasite *Plasmodium falciparum*, the bacterium *Helicobacter pylori*, and in certain human conditions such as rheumatoid arthritis and cancer. Dihydroorotate dehydrogenase (DHODH) catalyses the rate-limiting reaction in this pathway, and the enzyme has been well characterised as a target for chemotherapeutic intervention. Various *in silico* design and screening methods have been used by our colleagues in the School of Chemistry at the University of Leeds to identify potential inhibitors of both human and *P. falciparum* DHODH, and *in vitro* testing of these compounds has revealed that many of them bind to their target with high affinity. X-ray crystallography has been used to investigate the binding of some of these compounds to both human and *P. falciparum* DHODH, and the resulting high resolution structures have enabled us to rationalise the potency of our inhibitors, as well as faciliating the design of a second generation of compounds. *Helicobacter pylori* DHODH has been successfully expressed, purified and crystallised, and optimisation of the crystallisation condtions is ongoing.

Keywords: rational drug design, antimalarial, anticancer

MS16.P70

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Two-phase behavior of crystalline β-hematin: link to hemozoin (Malaria Pigment)

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Formation of crystalline hemozoin (HZ) is one of the main mechanisms in several blood-feeding organisms to detoxify free heme released upon digestion of host hemoglobin. Among these is the malaria parasite *P. Falciparum*, the most prevalent and most fatal among species affecting human beings. Although once considered eradicated, malaria has in recent times reemerged mainly due to parasitic resistance to commonly used quinoline drugs. These and other well-known antimalarials are anticipated to inhibit nucleation and crystal growth of HZ by binding to its surface[1], [2], [3]. Clearly, the crystallization process represents a vulnerability of the parasite, but the mechanism remains not fully understood.

Based on published spectroscopic and X- ray powder diffraction (XPRD) data, HZ is believed to be very similar to the synthetic compound β-hematin, which consists of heme moieties (ferriprotoporphyrin IX (Fe(3+) PPIX)) coordinated via Fe-O bonds to form cyclic centrosymmetric dimers [2]. However, acknowledging the enantio-facial symmetry of Fe(3+) PPIX, not only one, but four different Fe-O cyclic stereoisomers, two centrosymmetric and two chiral, of opposite handedness, should be formed in the crystallizing solution of β-hematin.

We address the question of the fate of the three other isomers and suggest the four isomers may all be present in different phases of β-hematin. A low-temperature (100 K) X-ray powder diffraction (XRPD) study of β-hematin was undertook and revealed the presence of not only the published phase, but also of a minor phase. Based on Rietveld refinement and DFT+vdW computations [4], we propose the minor phase consists mainly of the other centrosymmetric dimer in a crystal structure similar to that of the major phase. On symmetry grounds the two enantiomeric chiral isomers may be occluded into the growing crystals, introducing disorder. This occlusion may also explain the observed sub-micron size of the crystals; when the chiral dimers are adsorbed on the crystal faces, they would act as tailor-made additives, retarding crystal growth.

The existence of two phases in β-hematin stands in contrast to HZ, which according to published data crystallizes in only one phase. This finding may be essential for a better understanding and more complete determination of the crystal structure of HZ. From structural considerations the formation of a centrosymmetric dimer would imply the formation of the three other dimers. The formation of a chiral dimer of single-handedness is on the other hand unique. We therefore propose a bias towards the formation of one of the chiral dimers and thus HZ to consist primarily of chiral dimers. Such biasing could come about if the free heme was

restricted to react from one side only, i.e. if bound to molecular oxygen on one side.

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Keywords: hemozoin, bio-mineralization, x-ray_diffraction

MS16.P71

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Crystal structure of human RNase H2

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Human RNase H2 is a heterotrimeric enzyme involved in DNA replication and maintenance of genome stability. Mutations in any of the three subunits result in the development of Aicardi-Goutières Syndrome (AGS). Here, we report the crystal structure of human RNase H2 ABC complex at 3.1 Á resolution. Conformation of the catalytic subunit A resembles known structures of monomeric RNases H2 from archaea and bacteria, while the overall structure and arrangement of individual subunits in the complex is similar to the mouse RNase H2 structure. The B and C subunits form an intertwined dimer which makes contacts with two loops and the C-terminus of the A subunit. Human RNase H2 exhibits different substrate specificity and activity than bacterial RNases H2. Finally, we were able to map all 29 AGS-related mutations onto the structure thus providing insight into the molecular mechanisms underlying pathogenesis of this disease.

Keywords: RNase H2, crystal structure, disease

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Investigation of interactions involving organic fluorine in trifluoromethylated benzanilides

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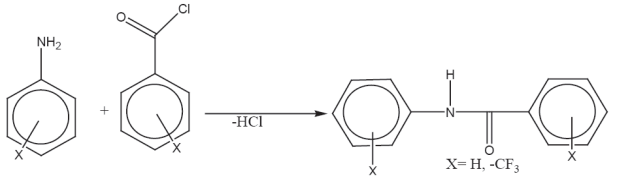
The importance of the C-F bond is now recognized with a large number of compounds (~20% of all pharmaceuticals and ~30% of all agrochemicals) containing organic fluorine. Inspite of its very high electronegativity, the involvement of organic fluorine in weak interactions has been extensively debated over a period of time. This feature has been ascribed due to its small size and very low polarizability [1]. Since the presence of organic fluorine in an organic molecule results in a modification in the chemical reactivity and biological activity, compared to its non-fluorinated analogue [2], it is imperative to understand interactions involving the fluorine atom. The formation of intermolecular O-H---F-C and N-H---F-C hydrogen bond were assumed important in the binding of the fluorinated compound to enzyme active sites [3]. In the last few years, the primary focus amongst the structural chemists has shifted towards the determination of crystal and molecular structures of drugs and pharmaceuticals containing organic fluorine [3].

In this regard, a library of *mono*- and *bis*-trifluoromethyl substituted benzanilides, have been synthesized and their crystal structures studied to investigate the nature of weak interactions involving the C(sp^3)-F bond. Benzanilides have been selected for this purpose due to the presence of -CO-NH- moiety which is an integral part of many drugs, biological molecules like amino acids, proteins etc. Crystallographic studies performed on a series of *mono*- and *di*- substituted fluoro benzanilide, containing C(sp^2)-F bond, shows mainly isosteric replacement of H-atom by F-atom along with the presence of C-H…F, F…F and C-F…π contact which dictate packing of molecules in the crystal lattice [4]. In comparison with fluoro substituted benzanilides, trifluoromethyl substituted compounds shows a wide range of crystal structures, crystallizing in triclinic, monoclinic, orthorhombic and the rare and unique tetragonal system. These crystallographic determinations are characterized by the presence of rotational disorder associated with the -CF_3 group. Investigation of their crystal structure shows presence of C-H…F-C(sp^3), lone pair…π, involving the fluorine atom along with the presence of strong N-H…O=C, weak C-H…O=C hydrogen bond and C-H…π interactions.



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Keywords: fluorine, intermolecular, conformation

MS17.P02

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Synthesis, crystal structure and theoretical calculations of Isonicotinaldehyde-N-phenylsemicarbazone and Biphenyl-4-carbaldehyde-N-phenylsemicarbazone

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Semicarbazones and their metal complexes are important classes of compounds which have long attracted attention, owing to their remarkable biological and pharmacological properties, such as antibacterial, antiviral, antineoplastic and anti-*Mycobacterium tuberculosis* activity [1]. Using the semicarbazone template was demonstrated, through a series of successive works, the significant anticonvulsant potential in epilepsy models for *aryl* semicarbazones [2].

In view of the importance of these compounds, two new semicarbazones (I) and (II) has been synthesized, and their crystal structures are reported here. Both semicarbazones molecules crystallize in a $P2_1/c$ space group. In the crystal packing the molecules are connected through N-H…O and N-H…N hydrogen bonds to form a centrosymmetric *synthon.* Other interactions like C-H…π and π…π stacking helps to stabilized the crystals.

The experimental geometries of the two compounds obtained from single-crystal X-ray diffraction were compared with those obtained from quantum-mechanical calculations. Theoretical calculations were performed by Gaussian03.