An *in silico* structure-based approach to anti-infective drug discovery

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SUMMARY

In light of the low success rate of target-based genomics and HTS (High Throughput Screening) approaches in anti-infective drug discovery, *in silico* structure-based drug design (SBDD) is becoming increasingly prominent at the forefront of drug discovery. *In silico* SBDD can be used to identify novel enzyme inhibitors rapidly, where the strength of this approach lies with its ability to model and predict the outcome of protein-ligand binding. Over the past 10 years, our group have applied this approach to a diverse number of anti-infective drug targets ranging from bacterial D-ala-D-ala ligase to *Plasmodium falciparum* DHODH. Our search for new inhibitors has produced lead compounds with both enzyme and whole-cell activity with established on-target mode of action. This has been achieved with greater speed and efficiency compared with the more traditional HTS initiatives and at significantly reduced cost and manpower.

Key words: *in silico* drug discovery, malaria, DHODH, bacterial resistance, RNA polymerase, D-ala-D-ala ligase.

INTRODUCTION

Infectious diseases such as malaria, tuberculosis and HIV are amongst the highest causes of morbidity and mortality worldwide (Wenzel and Edmond, 2000). Many of the gold-standard treatments for these diseases are under threat due to resistance factors and new medicines are urgently required to reduce this burden. Despite this, active anti-infective drug discovery programmes within the pharmaceutical industry have been on the decline since the 1990s.

Since the determination of the complete bacterial and eukaryotic genomes in the mid-1990s, drug discovery used a genomics-based approach whereby essential and conserved genes for the survival of a pathogen were identified and their corresponding proteins subjected to HTS to identify inhibitors. Unfortunately, this target-based genomic approach has failed to deliver the number of anti-infective drugs expected by the pharmaceutical industry (Livermore, 2011). Phenotypic screening has been at the forefront of anti-infective drug discovery for many years (Calderón *et al*. 2011) and the screening of large compound libraries is a long established method for the development of novel chemotherapeutics. However, this method of drug discovery only allows the investigation of a relatively small area of chemical space (López-Vallejo *et al*. 2012).

Structure-based drug design (SBDD) utilizes both the knowledge of three-dimensional protein structures and *in silico* techniques to identify putative small molecules with biological activity against desired protein targets (Stahl *et al*. 2006; Simmons *et al*. 2010). SBDD allows greater access to diverse areas of chemical space than compared to HTS, since *in silico* compound libraries can contain compounds not yet synthesized. Additionally, *de novo* design can allow access to all possible regions of chemical space (Simmons *et al*. 2010). Each method allows the development of novel chemotherapeutics outside the constraints of classical HTS.

Our research group uses the three main methods of SBDD (1) substrate-inspired design, (2) virtual high throughput screening (vHTS) and (3) *de novo* design of inhibitors. After the synthesis or purchase of the desired compounds they are subjected to biological evaluation to establish their level of inhibition against the target protein. Once hit compounds have been identified, further iterative rounds of design and optimization occur to improve binding affinity and physicochemical properties (Fig. 1). We will review the main computational tools for our *in silico* structure-based approach to novel anti-infectives and give examples of our successes in this therapeutic area.

COMPUTATIONAL TOOLS FOR SBDD

SPROUT (Gillet *et al*. 1994) is the main *de novo* design tool for the generation of ligands used by our

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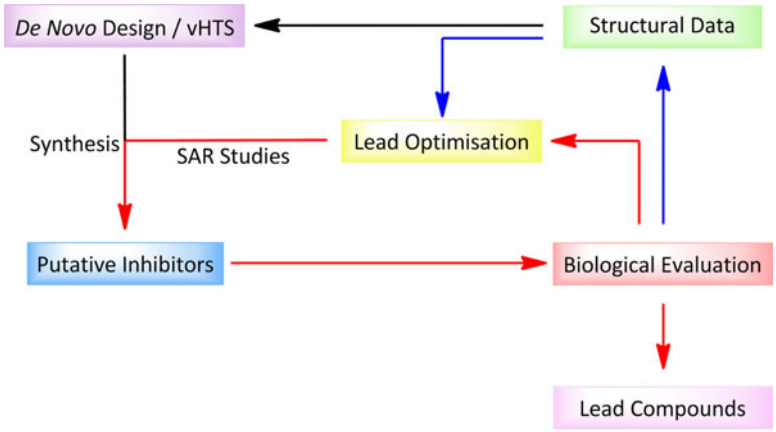

Fig. 1. From X-ray crystallography to lead compounds: an overview of the SBDD process.

research group, developed in-house by the Institute for Computer Applications in Molecular Science (ICAMS, University of Leeds, UK). The programme utilizes the principles of shape complementarity – the favourable fit of the three-dimensional shape of a molecule into the volume of a protein cavity coupled with optimal creation of binding interactions between the binding ligand and protein. Structure generation (Fig. 2) is achieved by firstly placing small fragments at chosen sites within the targeted binding region, each of which is placed to make a specific interaction with the protein. These molecular fragments are then joined in a stepwise fashion which satisfies the steric requirements, as well as the electrostatic and hydrophobic properties imposed by the protein to create the complete molecular scaffold. SPROUT can score the generated molecular scaffolds based on predicted binding affinity (calculated pKi of all the binding interactions) and/or molecular complexity (measure of synthetic accessibility).

SPROUT-HitOpt (Heikkila *et al*. 2007; Simmons *et al*. 2010) can be utilized once a hit compound has been identified in order to help guide the optimization of its binding interactions with the target protein. SPROUT-HitOpt operates via core extension whereby additional fragments or groups are added to structures representing the previously identified hit compounds so as to fill additional space and make additional interactions within the protein cavity. This software uses a retrosynthetic approach, utilizing a reaction database and a library of commercially available compounds from which alternative 'monomers' can be selected in order to extend the structure of the hit in ways which are compatible with its actual structure.

eHiTS (Zsoldos *et al*. 2007) is a flexible ligand docking and vHTS software package developed by SimBioSys Inc. This software uses an innovative exhaustive docking algorithm and consistently performs well in pose-prediction studies, where various docking programs are asked to reproduce co-crystal protein-ligand binding conformations. The docking algorithm searches all possible areas of space within the binding site. To achieve this, eHiTS splits the ligand(s) into smaller units consisting of rigid and flexible fragments, which are then docked into the three-dimensional space of the protein receptor in all possible configurations. The program then generates 'solutions' from the docked fragments that satisfy the steric constraints of the receptor surface (Fig. 3), which are ranked using the eHiTS scoring function.

AutoDock (Morris *et al*. 2009) is a docking software suite which predicts the ability of a small molecule to bind to a receptor. The AutoDock suite is a combination of three programs; AutoGrid, which calculates grid maps of interaction energies for the atom types present in ligands being docked, AutoTors, to identify and define rotatable bonds within the ligand, and AutoDock which utilizes a Lamarckian-Genetic algorithm to dock the ligands into the protein (Huey *et al*. 2007). Each module is manipulated through AutoDockTools which is the GUI for AutoDock, AutoTors and AutoGrid (Morris *et al*. 2009). AutoDock searches all possible ligand conformations within the binding site (Fig. 4). This technique should identify the lowest energy 'pose' a ligand can take within the binding site. AutoDock achieves this *via* a series of small conformational changes and larger conformational 'mutations' to the ligand to escape local energy minima in-order to find the most stable binding pose for a given ligand. It is important to note that this technique is more computationally intensive than the approach utilized by eHiTS resulting in much smaller numbers of compound structures being screened within a comparable time period.

Rapid Overlay of Chemical Structure (ROCS) (Grant *et al*. 1996) uses shape similarity to overlay a target molecule to a compound library to search for molecules that may have similar chemical and biological properties. ROCs operate under the assumption that compounds with similar three-dimensional shape and chemical properties to a known hit compound could show similar biological activity. ROCS compares molecular structures based

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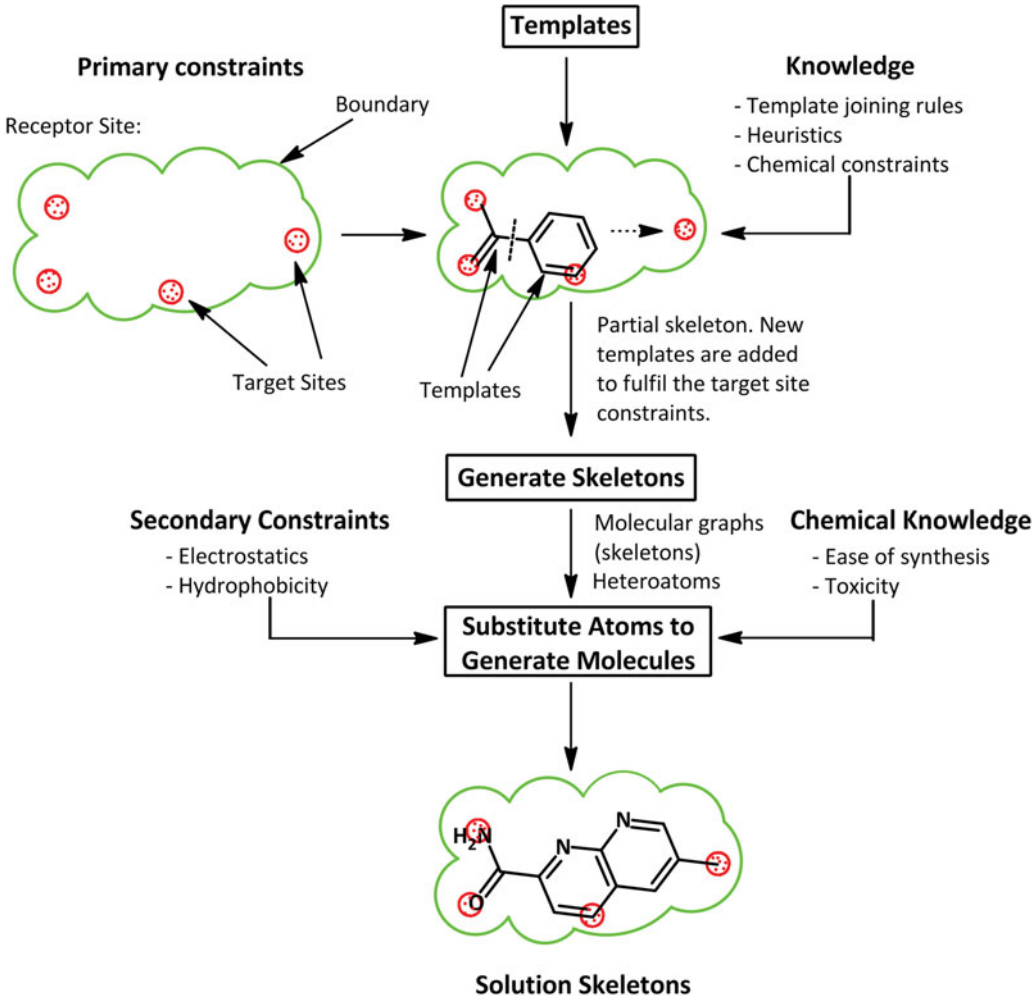

Fig. 2. Skeleton generation in SPROUT. First the primary constraints are defined by the boundary of the receptor site. Skeleton generation then places a template at the target sites until all sites are satisfied and no boundary violations have occurred. Finally, atoms are substituted in each skeleton to satisfy secondary constraints such as electrostatics and hydrophobicity.

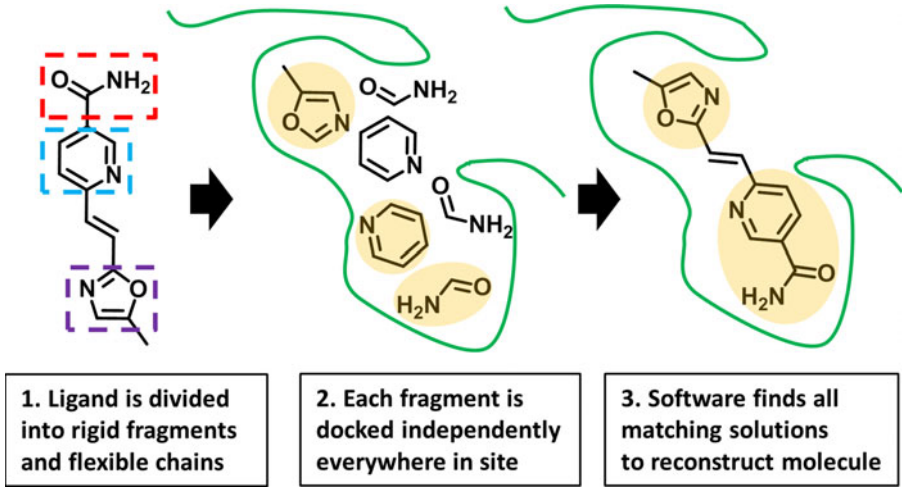

Fig. 3. The workflow of the eHiTS docking program.

upon three-dimensional shape where the software tries to maximize shared volume between the molecules, while an additional option allows the overlap of similar chemical properties/ electrostatics such as hydrogen bond donors or acceptors, cationic, anionic or hydrophobic groups.

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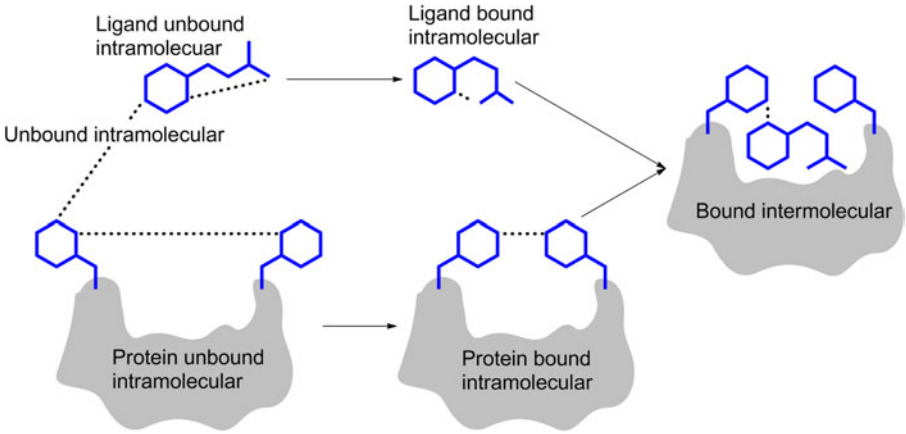

Fig. 4. Schematic of the AutoDock force field evaluating the free energy of binding in two steps. First, AutoDock calculates the intramolecular energetics of the transition of the ligand and protein from its unbound to bond conformation. Secondly, AutoDock evaluates the intermolecular energetics of combing the ligand and protein in their bound conformations. Adapted from (Huey *et al*. 2007).

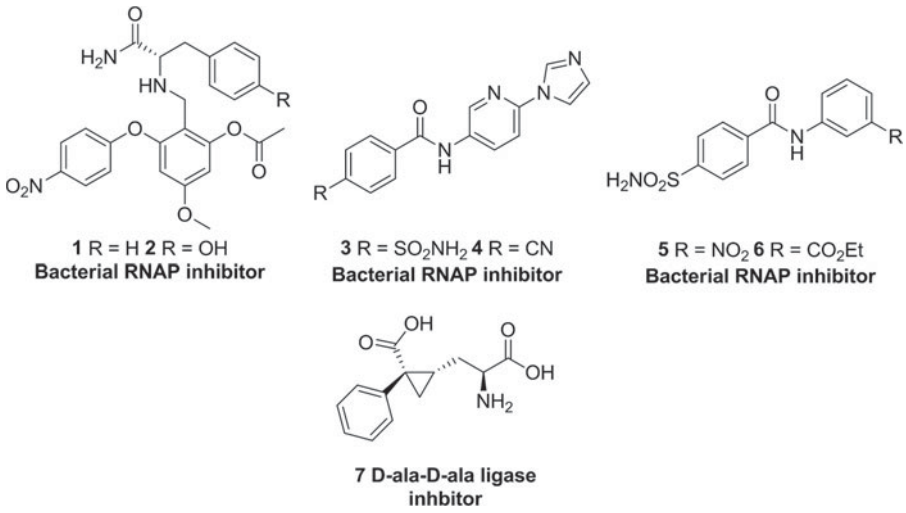

Fig. 5. Inhibitors of various bacterial enzymes designed using *in silico* methods by our research group.

RESULTS AND DISCUSSION

Example 1 – bacterial infections

During the golden age of anti-bacterial drug discovery between 1940 and 1962, over 20 distinct antibiotic classes were introduced to the market, but only three classes have been approved in the following 45 years: linezolid in 2000, daptomycin in 2003 and retapamulin in 2007 (Coates *et al*. 2011). This decline in productivity is in part due to dereplication of natural products and a failure to deliver new agents using HTS. Novel inhibitor classes are now being sought which have alternative modes of action to existing antibiotic classes. Our interest in combating bacterial resistance is focused on the bacterial targets, D-ala-D-ala ligase and RNA polymerase, respectively and here we summarize our work that has identified several series of new small molecule inhibitors (e.g. Fig. 5, **1–7**) of these enzymes.

RNAP polymerase. DNA-dependent RNA polymerase (RNAP) is a nucleotidyl transferase enzyme, essential for gene expression in all living organisms. It is the central enzyme in the transcription cycle, catalysing the production of RNA from a DNA template (Cramer *et al*. 2001). Despite its conserved function, RNAP does not share extensive structural homology amongst bacterial, archaeal, eukaryotic and viral RNAPs so enabling the design of selective therapies. This makes RNAP a very attractive drug target for anti-bacterial agents (Chopra, 2007), especially considering that its large size offers many distinct binding sites for small molecular inhibitors. Our research efforts have focused on targeting the rifamycin and myxopyronin B inhibitor binding sites, two natural products with discrete binding cavities within RNAP (Fig. 6).

The X-ray co-crystal structure of *Thermus aquaticus* RNAP containing rifampicin (PDB ID: 1I6V) (Campbell *et al*. 2001) was used to evaluate the

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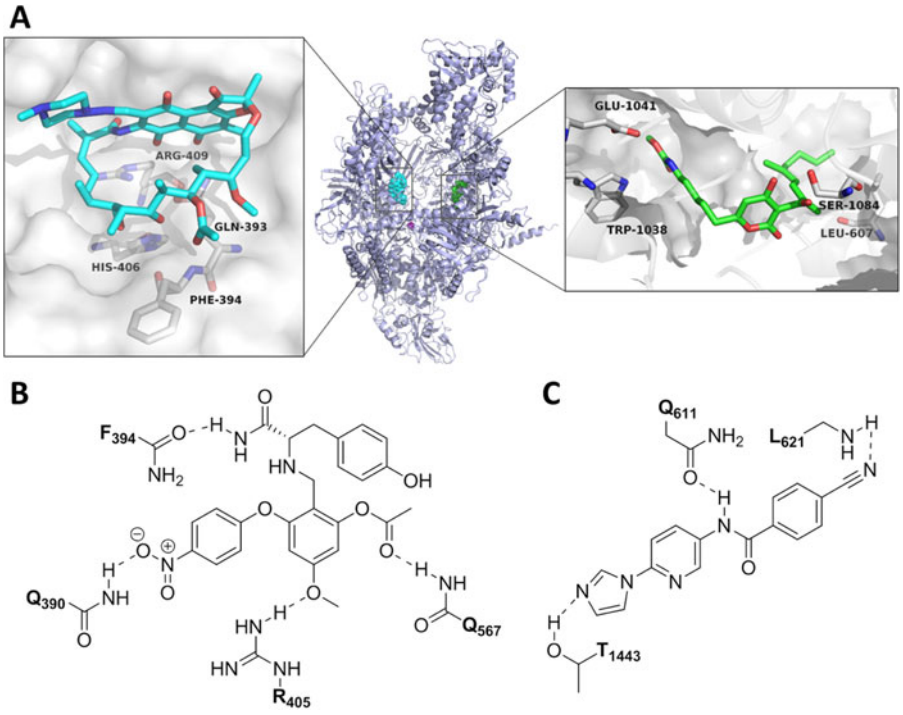

Fig. 6. (A) *Thermos thermophilus* RNAP with inserts of the rifamycin binding site (blue) and switch region (green), highlighting the binding modes of rifampicin and myxopyronin B respectively. (B) *De novo* designed inhibitor **2** targeting the rifamycin binding site, showing contacts to RNAP. (C) Switch region inhibitor **4** showing contacts to RNAP.

RNAP-rifamycin binding site, revealing a solvent-exposed cavity, approximately 12 Å away from RNAP's active site. Rifampicin makes several specific hydrogen bonds to RNAP (Fig. 6A) *via* the hydroxyl groups on the naphthalene ring and ansa bridge. When designing novel inhibitors for this site, we wished to utilize these RNAP residues, as well as additional regions of hydrophobicity located near the residues L391, L413 and F394. Application of the SPROUT software produced a synthetically attractive molecular skeleton based on a 1,2,4,6-tetrasubstituted aromatic core (Fig. 6B) (Agarwal *et al*. 2008). Several examples were synthesized and assayed for their ability to inhibit *Escherichia coli* RNAP using a SYBR Green assay (Daubendiek *et al*. 1995). Compounds **1** and **2** (Fig. 5) had IC_{50} values of 70 μM and 60 μM respectively, and represent the first examples of non-macrocyclic inhibitors that may inhibit RNAP at the rifamycin binding site. Further optimization of the scaffold is ongoing, as is an investigation into the mode of action of these compounds.

We have also applied *in silico* methods to the switch region of bacterial RNAP (Srivastava *et al*. 2011) in order to design new RNAP inhibitors. The switch region is a highly mobile unit of RNAP controlling the movement of the β' subunit and allowing double-stranded DNA to enter the active centre. Ebright *et al*. (Mukhopadhyay *et al*. 2008) showed that the structurally related antibiotics myxopyronin B and corallopyronin A, and the macrocyclic antibiotic ripostatin B bind to the switch region and inhibit bacterial RNAP with IC_{50} values less than 10 μM. At present, these antibiotics do not represent attractive lead candidates due to their unfavourable physico-chemical properties (high molecular weights and log*P* values) and narrow spectrum of antibacterial activity. After applying the SPROUT software to this cavity, and utilizing the hydrogen bond interactions made by myxopyronin B (Fig. 6B) to the protein, several new scaffolds were produced based on a benzamide core (McPhillie *et al*. 2011). Using the heteroatom substitution tool within SPROUT, a pyridyl-benzamide compound **3** (Fig. 5) was designed and following synthesis and biological screening, showed promising inhibition of *E. coli* RNAP (33% inhibition at 100 μM). Iterative rounds of optimization, guided by molecular docking, improved binding affinity for this scaffold, giving compound **4** (Fig. 6C) with an IC_{50} value of 7·2 μM.

The fragment-like compounds **5** and **6** (Fig. 5) also showed promising activity against RNAP at the micromolar level of inhibition. A structure-activity relationship study showed that *meta*-electron withdrawing groups on the *N*-phenyl ring maximized potency of the compounds (**5**: IC_{50} 23·3 μM, **6**: IC_{50} 5·6 μM). Indeed, both this class and the pyridyl-benzamide compounds proved to be selective for bacterial RNAP when assayed for inhibition of *S. cerevisiae* RNAP and the structurally unrelated enzymes, malate dehydrogenase and chymotrypsin (Seidler *et al*. 2003). Unfortunately, both series of

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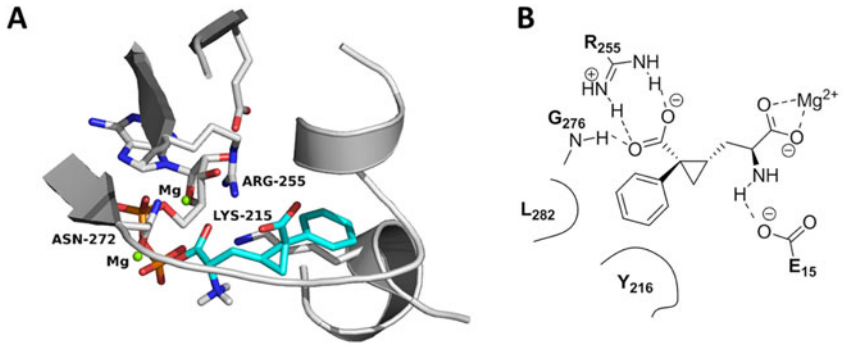

Fig. 7. (A) D-ala-D-ala ligase inhibitor **7** as designed using SPROUT in the enzyme active site. (B) Schematic diagram showing the inhibitor-protein interactions of inhibitor **7**.

compounds suffer from a lack of antibacterial activity against both Gram-positive and Gram-negative bacteria which we attributed to poor cell membrane penetration.

D-alaine-D-alanineligase. D-alanine-D-alanine ligase (Ddl) participates in peptidoglycan biosynthesis and catalyses the conversion of D-alanine to the D-ala-D-ala dipeptide, a substrate for the MurF enzyme. Inhibition of Ddl leads to a loss in cell rigidity and integrity, followed by cell death (Green, 2002). Therefore Ddl, as well as the peptidoglycan synthesis pathway, is an excellent target for novel anti-microbial agents. We used an *in silico* approach in order to identify new inhibitors of Ddl using SPROUT in conjugation with the *E. coli* Ddl crystal structure (pdb code: 2DLN) (Fan *et al*. 1994). A number of key residues involved in binding the phosphonate-based transition-state isostere were chosen as target sites, as well as an additional small hydrophobic pocket not utilized by the phosphonate inhibitor (Besong *et al*. 2005). A cyclopropyl-based amino acid **7** was selected for chemical synthesis as it satisfied the hydrogen-bond and steric requirements of the cavity (Fig. 7). Although **7** was synthesized as a 1:1 mixture of disastereomers, it displayed a K_i value of $12{\cdot}5 \pm 0{\cdot}1\ \mu$M against Dlb. The K_i value of cycloserine, a known inhibitor of Dlb, was $1{\cdot}4\ \mu$M, and therefore compound **7** is an excellent starting point for further optimization.

Example 2 – Malaria

Malaria is a global problem. Approximately 50% of the world's population is at risk of infection from the parasites which cause malaria (WHO, 2011), five of which are able to infect humans under normal conditions. The two parasites responsible for the greatest malaria burden are *Plasmodium falciparum* and *Plasmodium vivax* (White, 1999; WHO, 2011). Although there are myriads of current drugs used to treat malaria, unfortunately resistance to all current treatments has been observed. The development of resistance to all first-in-line treatments for malaria, coupled with the fact that the anti-malarial pipeline contains a very small number of novel chemical entities with novel modes of action (Olliaro and Wells, 2009), highlights an urgent requirement for the development of novel chemotherapeutics to combat this disease, particularly against strains which have previously developed resistance to earlier therapies.

Dihydroorotate dehydrogenase. Dihydroorotate dehydrogenase catalyses the fourth and only redox step of pyrimidine biosynthesis; the oxidation of dihydroorotate to orotate. This is achieved through the reduction of the resident enzyme co-factor FMN coupled with the oxidation of a respiratory quinolone such as co-enzyme Q_{10} (Christopherson *et al*. 2002). Interestingly, DHODH has been linked with the electron transport chain and it has been shown that its loss leads to mitochondrial dysfunction (Fang *et al*. 2012). Although humans also have an homologous DHODH enzyme, it has a low (26%) sequence similarity with *Pf*DHODH, and they are not dependent on this method for acquiring pyrimidine nucleosides. However, inhibition of the human homologue has been shown to have an immunosuppressive effect (Christopherson *et al*. 2002; Haque *et al*. 2002) and therefore it is important to avoid this undesirable effect in patients with an infection such as malaria.

De novo design of DHODH inhibitors. A detailed comparison of the active sites within the human and *Plasmodial* DHODH enzymes (from co-crystal structures 1D3H and 1TV5 respectively) (Liu *et al*. 2000; Hurt *et al*. 2006), containing the same bound inhibitor A77-1726 **8** (Fig. 8), revealed that the binding site in the human enzyme was 'flattened' compared to the less congested plasmodial binding site by the protrusion of $Ala_{Hs}59$ into the cavity (Fig. 9A) (Heikkila *et al*. 2006).

To exploit this structural difference we applied our *de novo* molecular design program SPROUT (Gillet *et al*. 1994) to the binding site of

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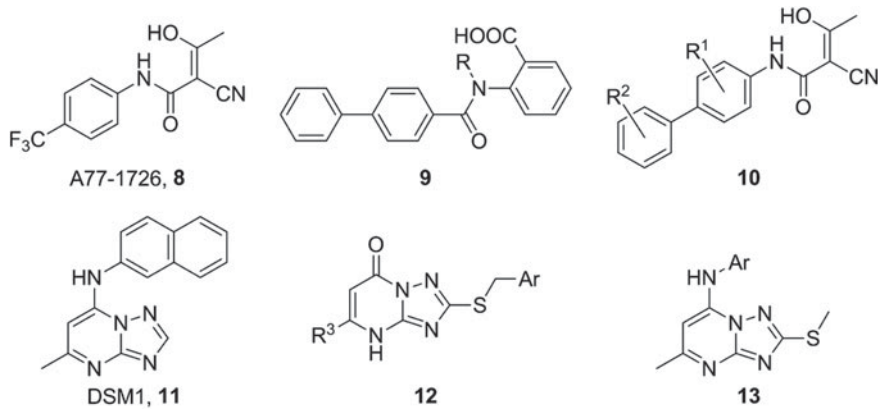

Fig. 8. Structures of the previously identified inhibitors of *Pf*DHODH A77-1726 **8** and DSM1 **11**, and the novel DHODH inhibitors developed by our group.

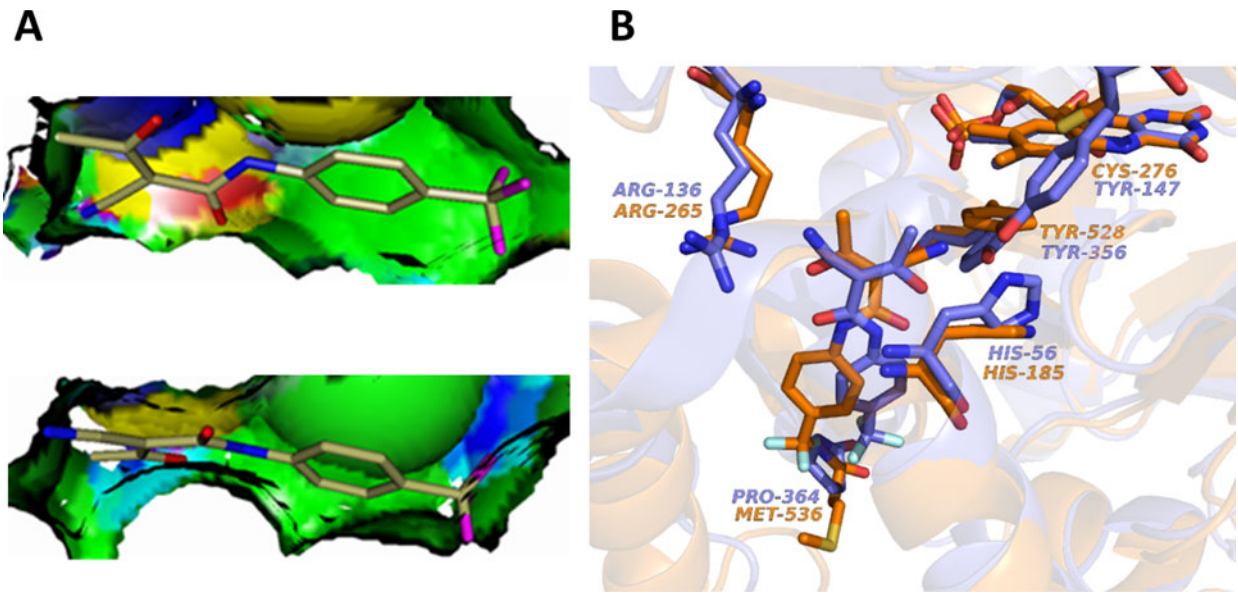

Fig. 9. (A) *Pf*DHODH binding site (top) with bound inhibitor A77-1726 **8**, showing the less congested nature of the *Pf*DHODH binding site. *Hs*DHODH binding site (bottom) with Ala59 protruding into the cavity and closing the hydrophobic binding site. (B) Overlay of the DHODH crystal structures 1TV5 (orange) and 1D3H (blue), showing the key residues in each binding site.

the co-crystal structure of *Pf*DHODH (1TV5, (Hurt *et al*. 2006)). In order to design structurally simple inhibitors, only two direct hydrogen bonding interactions were specified ($Arg_{Pf}265$ and $His_{Pf}185$), resulting in an anthranilic acid scaffold **9** (Fig. 8). A series of inhibitors were synthesized and tested against both *Pf* and *Hs*DHODH (Heikkila *et al*. 2006) in a colorimetric DCIP assay (Hines *et al*. 1986), the most potent of which, compound **9** (R = Me), was selective for *Pf*DHODH (IC_{50} value of $42{\cdot}6 \pm 4{\cdot}6\,\mu$M) over *Hs*DHODH ($IC_{50}$ value of $200\,\mu$M). It was assumed that this selectivity could be attributed to the non-planar conformation of the molecule induced by the presence of the methyl group on the amide nitrogen (Fig. 10B). This hypothesis was reinforced by the reduced activity of unsubstituted analogue compound **9** (R = H) against *Pf*DHODH ($153{\cdot}5 \pm 13{\cdot}2\,\mu$M) and its preferred binding to *Hs*DHODH ($5{\cdot}0 \pm 1{\cdot}6\,\mu$M).

Despite significant research we were unable to improve the potency of this compound series further, although the discovery that the selectivity between species could be achieved *via* the use of conformational staggering between adjacent aryl rings would be applied to later compound series.

Substrate-inspired structure-based drug design. A77-1726 **8** (Fig. 8) (Bruneau *et al*. 1998; Heikkila *et al*. 2007; Davies *et al*. 2009) is a weak inhibitor of *Pf*DHODH but the co-crystal structure of this compound with *Pf*DHODH offered a good starting point for substrate inspired structure-based drug design of inhibitors (Hurt *et al*. 2006;

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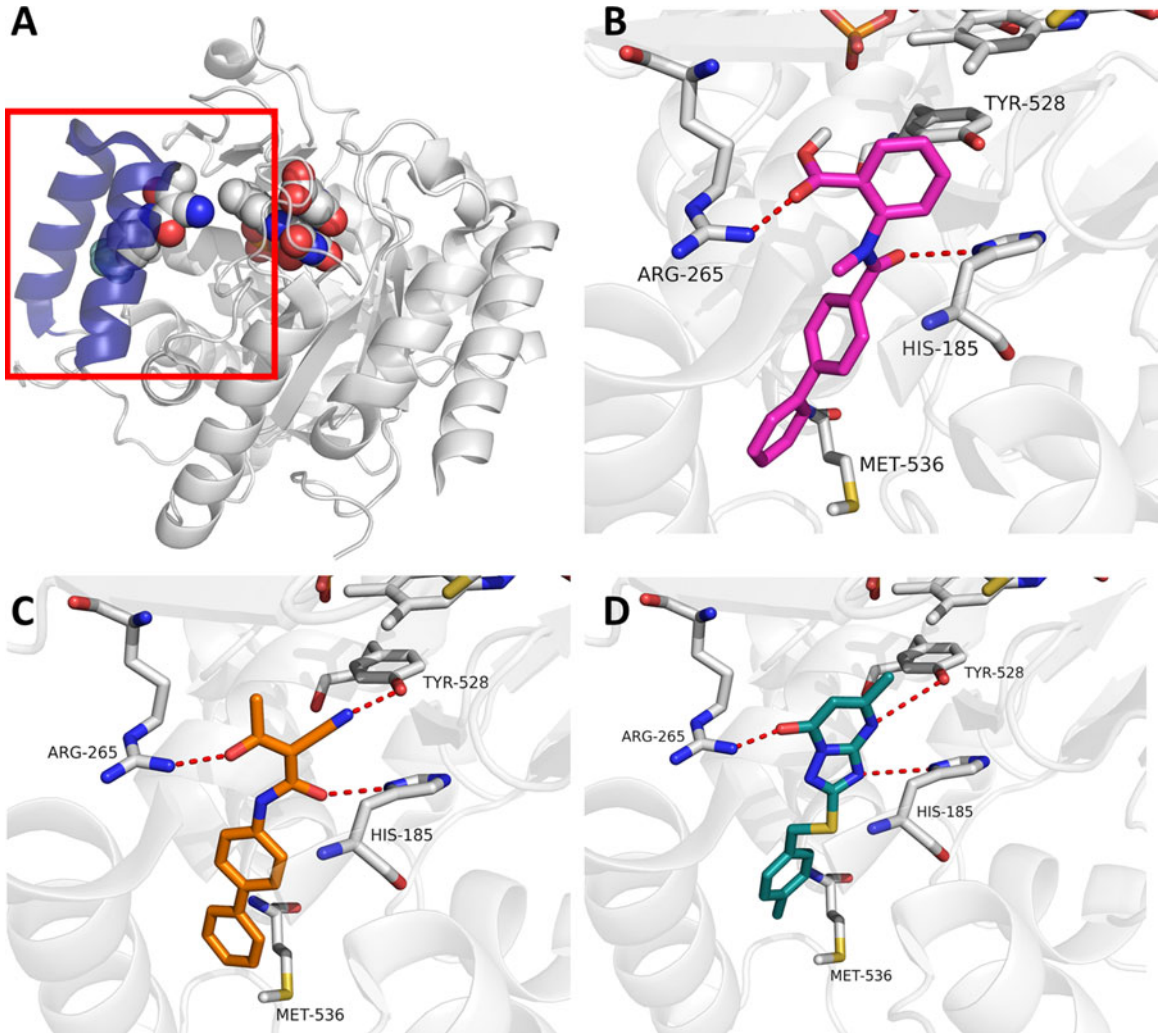

Fig. 10. *In silico* dockings for the designed inhibitors in *Pf*DHODH synthesized by our research group. (A) *Pf*DHODH co-crystal structure (1TV5), indicating the location of the substrate binding site (red box). (B) anthranilic acid derivative **9** (R = Me) bound in *Pf*DHODH (1TV5). (C) A77-1726 inspired compound **10** (R^1 & R^2 = H) bound in *Pf*DHODH (1TV5). (D) *S*-benzyl series 12 identified *via* shape similarity screens based on DMS1 **11** bound in *Pf*DHODH, from co-crystal structure 3I68.

Heikkila, 2007). Further examination of the human and *Plasmodium* co-crystal structures containing this inhibitor revealed several additional differences to those previously discussed. The hydrogen bonding network for the bound inhibitors is different in both enzymes; in *Pf*DHODH there are three distinct hydrogen bonds to $His_{Pf}185$, $Arg_{Pf}265$ and $Tyr_{Pf}528$ whereas in the *Hs*DHODH complex there are two water-mediated hydrogen bonds to $Arg_{Hs}136$ and $Gln_{Hs}47$ in addition to a direct interaction with $Tyr_{Hs}356$ (Fig. 9B).

The SPROUT-HitOpt program (Simmons *et al*. 2010) was utilized to generate variants of the bound inhibitor **8**. We opted to retain the hydrogen bonding network and 'head group' of **8**, whilst optimizing the aromatic 'tail' as it was reasoned that the difference in shape and hydrogen bonding networks of the binding site in each enzyme would allow for the development of species-selective inhibitors (Heikkila *et al*. 2007). A series of inhibitors utilizing substituted biphenyls as extensions of the hydrophobic 'tail' of **8** was synthesized (Fig. 8, **10**). As we observed in our previous work that the *Pf*DHODH binding site was better suited to non-planar groups in the hydrophobic pocket of the binding region, substituents were placed in the ortho-positions of the rings to 'lock' them into an orthogonal conformation. Unfortunately, these compounds were only weakly active against the *Pf*DHODH enzyme (4·0 μM, R^1 & R^2 = Cl, 10). It is important to note that some of these inhibitors displayed potent inhibition of the *Hs*DHODH with IC_{50} values as low as 22 nM (R^1 & R^2 = Cl, 10).

In silico docking studies of these compounds in the *Pf*DHODH binding site indicated that the residue $Met_{Pf}536$ at the entrance to the binding cavity would clash with the phenyl ring of the inhibitors (Fig. 10B). The human enzyme has the much smaller $Pro_{Hs}364$ residue at this position which is small enough not to sterically clash with the inhibitors,

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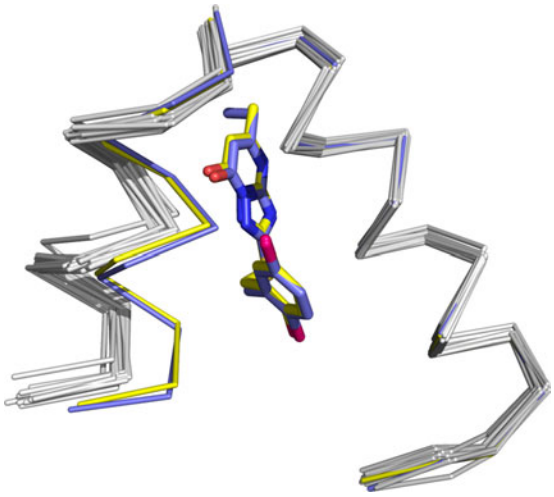

Fig. 11. Overlay of all previously published *Hs*DHODH co-crystal structures (grey), and the two newly obtain *Hs*DHODH co-crystal structure (blue and yellow), highlighting the structural shift in the $\alpha-1$ helices of this enzyme.

consistent with the good observed inhibition of this enzyme (Hurt *et al*. 2006).

We were subsequently able to obtain several X-ray co-crystal structures of these inhibitors bound within *Hs*DHODH which helped confirm these predictions. Additionally, it was observed that the residue $His_{Hs}56$ (homologous to $His_{Pf}185$) was unable to hydrogen bond to these inhibitors, even when they adopt a similar orientation to that observed for A77-1726 **8** bound within *Pf*DHODH (Fig. 9B). Instead, $His_{Hs}56$ is seen to hydrogen bond to $Tyr_{Hs}147$. The homologous residue to $Tyr_{Hs}147$ in *Pf*DHODH, $Cys_{Pf}276$, is unable to form this hydrogen bond resulting in $His_{Pf}185$ and $His_{Hs}56$ adopting different conformations in each enzyme respectively and more importantly, removing the possibility of $His_{Hs}56$ to form hydrogen bonds with a bound inhibitor (Fig. 9B). This observation coupled with previously published mutagenesis studies, where mutation of $His_{Pf}185$ resulted in the loss of tight binding for some selective inhibitors of *Pf*DHODH, showed that in order for a compound to be selective for *Pf*DHODH over *Hs*DHODH, a hydrogen bond to $His_{Pf}185$ is required (Heikkila *et al*. 2007). Since publishing our findings, a number of other studies have highlighted the importance of $His_{Pf}185$ for selective binding to *Pf*DHODH (Bedingfield *et al*. 2012).

Shape similarity screening. In 2008 Phillips *et al*. reported the structure of a series of potent and selective triazolopyrimidine inhibitors (**11**, Fig. 8) of *Pf*DHODH discovered using HTS (Phillips *et al*. 2008). Based upon this work, we identified compound **12** (Fig. 8, **12**, R^3 = Me, Ar = 4-chlorophenyl) as a moderately selective inhibitor of *Pf*DHODH, with an IC_{50} value of 1·0 μM (IC_{50} value of 39 μM against *Hs*DHODH) from a shape similarity search, using ROCS with compound **11** as the template and using the Maybridge chemical screening library.

Several analogues were subsequently synthesized and tested against both *Hs* and *Pf*DHODH. However, no improvement in the inhibition of *Pf*DHODH was found compared with **11**. Interestingly, *S*-benzyl triazolopyrimidine compound **12** (R^3 = Me, Ar = 2,5-dichlorophenyl) was a potent inhibitor of *Hs*DHODH (51 nM) and was subsequently crystallized with *Hs*DHODH revealing a small hydrophobic pocket adjacent to the pyrimidine methyl group. The extension of the methyl group to an ethyl group maximized these hydrophobic interactions and improved the IC_{50} value to 13 nM (when R^3 = Et, Ar = 2,5-dichlorophenyl). Both methyl and ethyl *S*-benzyl triazolopyrimidine derviatives were also crystallized in *Hs*DHODH revealing a previously unseen conformational change in the two α-helices enclosing the respiratory quinone binding site (Fig. 11) (Bedingfield *et al*. 2012). *In silico* studies of the predicted binding of these compounds within *Pf*DHODH suggested that they adopt a similar binding pose to that observed in *Hs*DHODH (Fig. 10). Mutagenesis studies on both *Pf*DHODH and *Hs*DHODH once again confirmed the observation that His*Pf*185 is a key residue for the binding of selective inhibitors in the *Pf*DHODH binding site (Bedingfield *et al*. 2012).

Most recently, *in silico* modelling based upon the *S*-benzyl series of inhibitors (Fig. 10D), has resulted in the production of a subsequent series of compounds which show excellent inhibitory activity of *Pf*DHODH, and potent anti-plasmodial activity. Full details of this new series will be published elsewhere.

CONCLUSIONS

SBDD utilizes knowledge from three-dimensional protein structures and applies *in silico* techniques to identify putative small molecules with biological activity against desired protein targets. We have applied these methods to enzymes such as dihydroorotate dehydrogenase, bacterial RNAP and bacterial D-ala-D-ala ligase, and successfully developed compounds which not only inhibit these enzymes but, in the case of dihydroorotate dehydrogenase, are potent against the infecting pathogen. The use of *in silico* modelling has helped us develop species-selective inhibitors by harnessing subtle differences within the binding site to infer selectivity. This is one of the many advantages of SBDD, that selectivity can be built into a series of molecules from an early stage helping to minimize side effect and undesirable effects *in vivo*.

Our structure-based approach emphasizes the importance of structural molecular genomics and

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validates its use within the field of anti-infective drug discovery. It offers rapid progression from target to lead compound at a reduced cost compared to the standard approaches to drug discovery which focus on HTS. Exploiting these tools is essential for anti-infective drug discovery to combat the rise in resistance to front-line therapeutics. Advances in both the development of computational hardware and in the more advanced SBDD algorithms will continue to increase the attractiveness of using this technique to aid drug discovery of anti-infectives. However, the major challenge here is the design of drug-like compounds capable of entering (and remaining within) the cell of the pathogenic organism.

SOFTWARE ACCESSIBILITY

SPROUT, SPROUT-HitOpt and eHiTS software are maintained by SimBioSys Inc. and further information can be obtained *via* the Symbiosys website; http://www.simbiosys.ca. Autodock is freeware for academics and can be accessed at http://autodock.scripps.edu. ROCS is maintained by OpenEye and is available from http://www.eyesopen.com/rocs.

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