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Applications of structure-based design to antibacterial drug discovery

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ABSTRACT

In recent years bacterial resistance has been observed against many of our current antibiotics, for instance most worryingly against the cephalosporins which are typically the last line of defence against many bacterial infections. Additionally the failure of high throughput screening in the discovery of new antibacterial drug leads has led to a decline in the number of antibacterial agents reaching the market. Alternative methods of drug discovery including structure based drug design are needed to meet the threats caused by the emergence of resistance. In this review we explore the latest advancements in the identification of new antibacterial agents through the use of a number of structure based drug design programs.

1. Introduction

1.1. Antibacterial agents

The emergence and spread of antibiotic resistant bacteria is a serious threat to world health [1]. The development of new antibiotics with novel modes of action is essential in order to provide replacements for current drugs for which resistance is widespread [2]. The increase in human air travel over recent years and the unregulated prescription of antibiotics in the developing world are believed to be important factors in the rapid spread of antibiotic resistant bacterial strains. The discovery of new antibiotic classes has proven to be a serious challenge with no entirely new classes identified since the discovery of the lipopeptide antibiotic Daptomycin in 1987 [3]. Many of the older antibiotics classes are derived from natural products, but in the 1980s and 1990s, as the discovery of new natural products with antibiotic properties became more challenging, the development of technologies such as combinatorial chemistry, genomics and high throughput screening (HTS) encouraged pharmaceutical companies to move away from searching for natural products and towards looking for small molecule inhibitors. To date these approaches have proven less successful at providing lead compounds for antibacterial targets as they have been in other disease areas [4]. Computational strategies to aid the discovery of new antibacterial agents are now commonly employed to compliment more traditional medicinal chemistry approaches [5]. These strategies are discussed below.

1.2. Structure based drug design (SBDD)

Identifying a suitable target is the first consideration when starting any structure based drug design program. An antimicrobial-drug target should be essential, have a unique function in the pathogen and exhibit an activity that can be altered by small molecules. The small molecules should be specific for the bacteria and therefore be nontoxic. A small number of programs including SiteMap [6] are available which help to identify potential binding sites within a given protein [7].

Most SBDD programs rely upon a single high-resolution crystal structure. It is however important to take into account the fact that a protein when in solution is flexible and will often undergo conformational changes upon substrate binding. A number of studies have shown the importance of allowing both the protein and the ligand to have free movement in binding [8]. Examining protein flexibility also rapidly increases the computational time to model ligand binding and therefore most programs will only simulate ligand flexibility. Molecular dynamics studies can be conducted on proteins to identify flexible binding regions, however the complexity and time of calculation makes this approach inefficient and unrealistic for large compound libraries.

The Protein Data Bank [9] is often the source of many known structures. However, for a large number of new targets, crystal structures are unavailable and therefore construction of a homology model is often necessary providing that a crystal structure is available for a protein with substantial sequence similarity [10]. A number of programs including SWISS-MODEL [11] and PHYRE2 [12] have been designed to automate the process of making a homology model.

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There are three main methods used to identify new ligands based on structural information. The first approach is 'substrate-and known inhibitor design' where a known inhibitor or co-factor is modified to improve binding affinity. In the second approach, *de novo* design, a molecule is designed from scratch to bind in the active site. Fragments are docked into sub-sites, and then joined to create full molecules. These molecules are scored and ranked *in silico* based on the interactions present with the target protein. The third approach is the docking of small molecule libraries into the desired target crystal structure. Molecules are ranked using a scoring function. There are a number of programs available to perform such 'virtual high throughput screening' (vHTS). Recent examples of these three approaches are discussed below.

2. 'Substrate-and known inhibitor design'

This approach takes a known inhibitor and structurally modifies it to give more potent inhibitors. Examples of programs which have been employed in this area include SPROUT HitOpt [13] and Maestro [14].

Maestro [14] is part of the Schrödinger software package and allows users to visualise the desired receptor in three-dimensional form. A surface of the molecule can be generated and any areas for possible expansion or modification of the inhibitor can be identified and modified within the package. The resulting inhibitor can be re-docked using the Glide module an add-on of the maestro package (see Section 4.3). Work by Wang et al. looked at the inhibition of glycoside hydrolases using Maestro. Although not a bacterial target, the inhibition of glycoside hydrolase has widespread application in the treatment of diabetes, viral infections, lysosomal storage diseases and cancers. Using a transition state mimic strategy, a series of C2-substituted gluco-configured tetrahydroimidazopyridines were designed and synthesised. Maestro was used to study the structure–activity relationships by docking the compounds into the beta-glucosidase active sites. Each structure was then scored with the Glide scoring function aiding the design of more potent ligands, including **1** (Fig. 1) [15].

SPROUT-HitOpt (formally SPROUT-LeadOpt) builds on the SPROUT program (see Section 3.1). SPROUT-HitOpt is designed to produce structures that are similar to known lead molecules and have improved predicted binding affinities. SPROUT-HitOpt has two main functions to aid lead optimisation. The first is core extension which allows the user to explore different regions of a protein binding site by adding fragments, via a set of synthetic rules, to a core molecule. SPROUT-HitOpt identifies suitable functional groups on the core molecule and extends the structure from this point using a combinatorial library of commercially available compounds. The second function is monomer replacement which uses retrosynthetic fragmentation to identify monomers within one or more bound ligands. These monomers are then replaced in combinatorial fashion by structurally related molecules to create new molecular structures, which can then be scored based on predicted binding affinity and synthetic accessibility.

A substrate-inspired approach was recently used to design compounds which inhibit enoyl reductase (ENR). ENR catalyses the rate-limiting step in the fatty acid biosynthesis pathway in bacteria and protozoan parasites, and is the molecular target for the antibacterial agent triclosan. Triclosan **2** (Fig. 1) is a transition-state isostere of the natural fatty acid substrate, where the phenol group mimics that enolate transition-state [16]. Much research activity has been dedicated to derivatives of the triclosan scaffold but the groups of Rice and McLeod have used X-ray crystallography to design triclosan derivatives which further mimic the fatty acid substrate [17], extending the triclosan scaffold out of the previously enclosed hydrophobic cavity within the binding site, generating potent inhibitors including **3** (Fig. 1).

3. *De novo* design

The first examples of software produced specifically for *de novo* drug design were reported in the early 1990s, and the development of new and improved programs has not stopped since [18]. The continued improvement in computing power and the increased availability of high-resolution protein crystal structures has made the use of such software increasingly attractive. A general description of the steps involved in *de novo* design is given in Scheme 1.

Chemical space, the total number of possible small organic molecules, is estimated to exceed 10^{60} [19]. When trying to design new molecules from scratch it is possible to come up with almost infinite possibilities. Therefore, the design of molecules must be constrained to those which fit certain spatial and electronic characteristics which will allow them to bind favourably to a target protein. The first step carried out by many *de novo* design programs is the analysis of the target site of the desired protein. Some programs, such as SPROUT [20] and LUDI [21], identify regions where favourable interactions could form between an appropriately functionalised ligand and the protein by looking for hydrogen bond donors and acceptors as well as hydrophobic residues in the protein structure. Other programs including GRID [22] and LigBuilder [23] use a grid-based approach moving probe fragments around a grid of points in the binding site and calculating the energy at each position to determine regions where favourable interactions might occur.

Once a binding site has been identified and analysed, molecular building blocks or fragments need to be chosen to begin designing full molecules. The type of building blocks used varies from program to program. Many programs, including CombiSMoG [24] and LigBuilder [23], have a database of small, simple fragments such as aromatic rings and linker fragments such as amides or alkyl chains which can be combined to give a wide range of structures. Other programs (AutoGrow [25] or SYBYL [26]) can search databases of compound structures and break them down into fragments which can be docked into the protein binding site.

The ways in which building blocks are connected also vary immensely. Some programs start from a single fragment positioned in the binding site and grow out from there by directly linking fragments to the start fragment one at a time, examples of this approach include BOMB [27]. Others start with several building blocks placed in favourable positions in the binding site and then connect them using linker fragments, e.g. SPROUT [20]. Once an appropriately sized small molecule has been produced, further improvements to the structure might be carried out by evolution or substitution at various positions.

An essential part of any *de novo* design software is a method for determining the relative binding affinity of the designed structures. Most programs contain a method for calculating the free energy of the designed structures within the protein binding site as a way of predicting the binding affinity and thus determining which compounds designed by the program are the most promising candidates. Structures generated using *de novo* design can be complex and difficult to synthesise, so many *de novo* design packages include a method for analysing the complexity or synthetic tractability of the designed structures.

Although a number of different software packages have been mentioned in the above discussion, this is not exhaustive and several detailed reviews covering the different software tools available have been published. These provide a more detailed account of the various techniques used and their application to different areas of

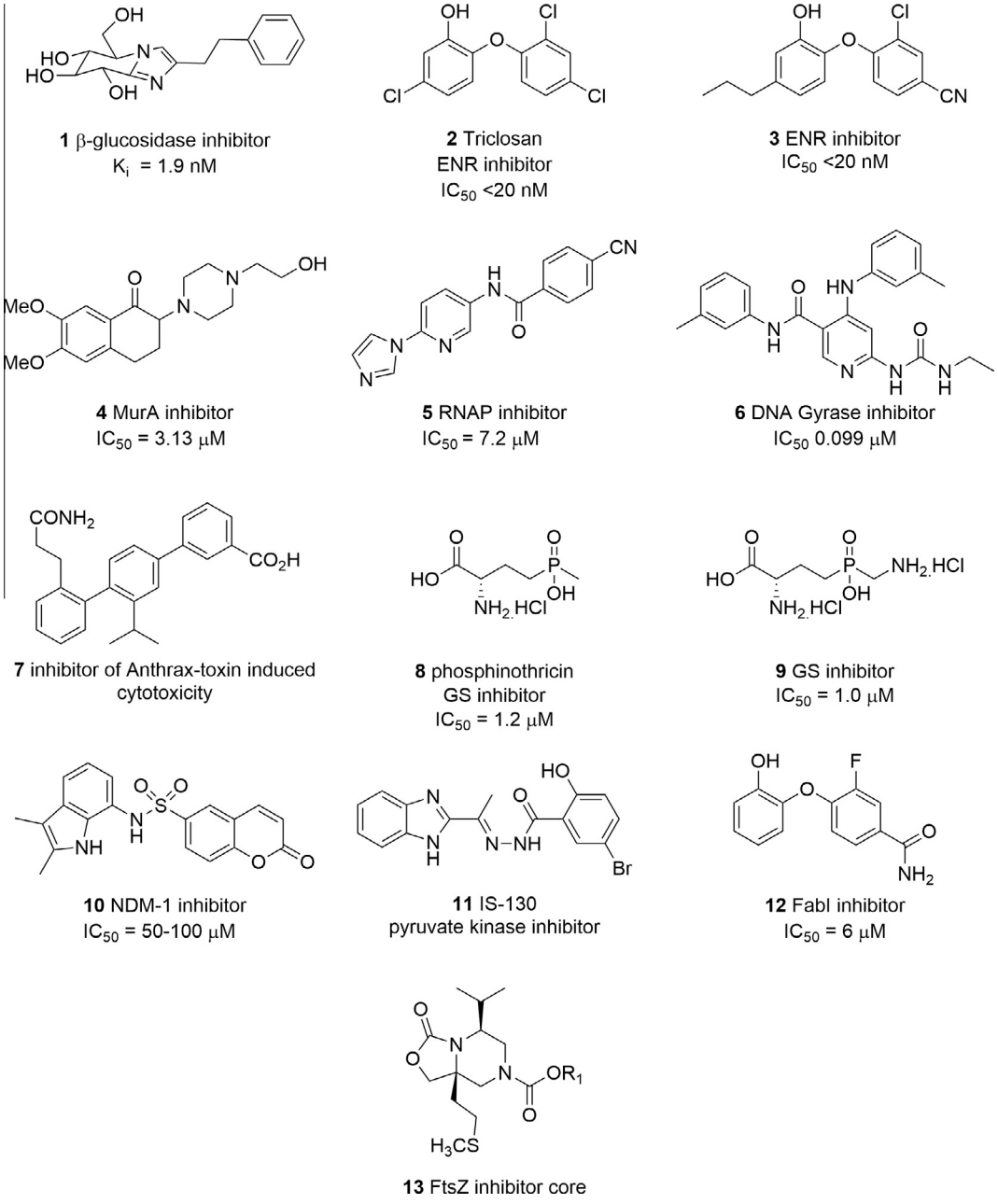
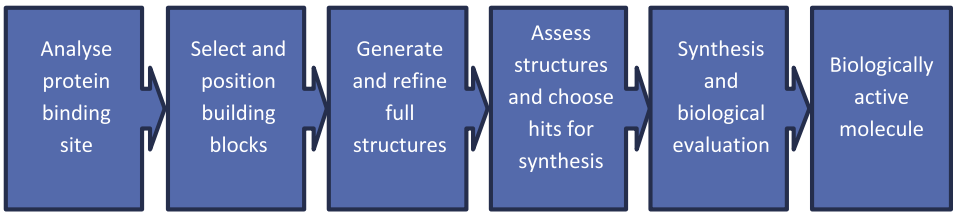


Fig. 1. Inhibitors designed using structure based drug design.



Scheme 1. General summary of the *de novo* design process.

drug discovery [18,28,29]. Specific examples of the use of some of these in antibiotic discovery are presented below.

3.1. SPROUT

In recent years, SPROUT has been the program most frequently used in the design of inhibitors of antibacterial targets [30–33].

SPROUT is made up of five modules CANGAROO, HIPPO, ELEFANT, SPIDER and ALLIGATOR, each concerned with a different part of the *de novo* design process (Scheme 2). CANGAROO is concerned with identification of a binding site in the crystal structure of the target protein. HIPPO identifies hydrogen bond donors and acceptors and hydrophobic regions within this binding region and allows the user to select key interactions. ELEFANT allows selection of molecular

fragments to be docked into each of the target sites. These fragments are then connected using spacer templates in SPIDER to give inhibitor 'skeletons' which can undergo heteroatom substitution and be sorted by predicted binding score and complexity to give the most promising candidate structures using ALLIGATOR.

Since 2000, a main focus of work in the Fishwick/Johnson group at Leeds has been on the design of inhibitors of peptidoglycan biosynthesis. Work had concentrated initially on the design of inhibitors of the Mur enzymes, MurA-F. Whilst some earlier success was found with macrocyclic inhibitors of MurD [30], it proved very difficult to produce molecules with the potency required to gain whole cell antibacterial activity (unpublished results). An exception to this was seen with a series of 2-aminotetralone inhibitors, including **4** (Fig. 1), of MurA, whose predicted binding mode was modelled using eHiTS [31]. Other inhibitors of peptidoglycan biosynthesis enzymes such as penicillin-binding proteins have been designed using SPROUT in collaboration with a number of other academic groups [32].

Bacterial RNA polymerase (RNAP) is another target which has been the focus of extensive research involving both the Chopra and Fishwick groups at the University of Leeds [34]. A validated molecular target, RNAP is the main component of transcription, synthesising RNA from a DNA template. Several natural products inhibit this multisubunit enzyme at discrete binding sites, which have been used as potential receptor sites in the *de novo* molecular design of novel small molecule inhibitors. Several SPROUT designed compounds have been confirmed to be RNAP inhibitors including compound **5** (Fig. 1) [33]. A lack of cellular potency displayed by the compounds was attributed to problems penetrating bacterial membranes.

More recently, SPROUT has been used to design novel dual-inhibitors of the bacterial type II topoisomerases DNA gyrase and topoisomerase IV [35]. Inhibitors of the ATPase site have been under investigation for some time and there are many published examples [36–38]. A common feature in many inhibitor series is an *N*-ethyl urea group adjacent to a heteroaromatic ring [39]. The ethyl urea fragment was used as the starting point for a SPROUT investigation. Four 'target sites' within the active site were selected and the *N*-ethyl urea motif was docked into the "target sites" for Asp 82 and Arg 86 (Fig. 2A). Fragments were docked into the two remaining target sites and the fragments connected to give 711 molecular scaffolds. These were assessed for their structural complexity, predicted binding score and synthetic tractability. Compounds based on the three most promising scaffolds were synthesised and the compound with the best antibiotic activity, the pyridine carbamate compound (Fig. 2B), was chosen for further development and full SAR. Following optimisation of the *de novo* hit compound, a very promising series of inhibitors was developed including **6** (Fig. 1) which had an IC_{50} of 0.099 μM and an MIC value against *Staphylococcus aureus* of 1 μg/mL [40].

3.2. CAVEAT

CAVEAT is a program that can be used both for *de novo* design and for the development of mimics of a known ligand. It focusses on the orientation of bonds rather than the position of atoms. Large databases of compound structures can be searched rapidly using this software to look for compounds that match a set of bond vectors described by the user. This is then used as a basic scaffold which can be modified to increase the compounds binding affinity for the target protein [41]. Two databases (ILLIAD and TRIAD) of simple, computer generated, hydrocarbon scaffolds have been designed to provide starting points for design of compounds using this software [42].

CAVEAT has recently been used to design small molecule inhibitors of the anthrax toxin protective antigen (PA) which heptamerises within the membranes of cells in the infected organism allowing transmission of other toxins into the cell. Residues on the surface of the protein were identified and structures designed which would bind to these and prevent a protein–protein interaction between monomers of PA [43]. Several of the designed compounds, including **7** (Fig. 1) displayed an ability to inhibit the toxicity of the anthrax toxins protective antigen and lethal factor in a cell based assay. Although not strictly antibiotics, compounds of this type would be protective against cellular damage caused by infection with anthrax bacteria.

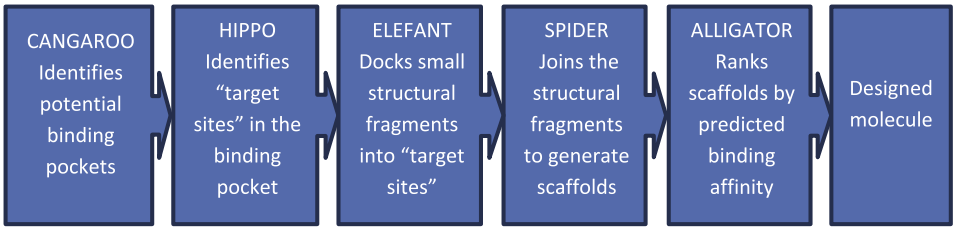
3.3. LUDI

LUDI is a module in *Discovery Studio* [44]. It works in a similar manner to SPROUT, beginning with analysis of the binding site, then docking small fragments into favourable positions in the binding site before connecting the docked fragments using linker fragments or atoms [21]. LUDI can also be used to modify or extend a fragment of interest by docking it into the receptor and using it as a starting point for growth of a structure.

LUDI has recently been used in the design of derivatives of phosphinorthricin **8** (Fig. 1) as inhibitors of glutamine synthetase [45]. A crystal structure of glutamine synthetase complexed with phosphinorthricin indicated that binding was quite tight and there was only limited opportunity for derivitisation. However, LUDI was able to identify target sites near the methyl chain of phosphinorthricin **8** (Fig. 1) and suggested several derivatives of which 6 were synthesised and screened for activity. All were found to be low micromolar inhibitors of the enzyme and compound **9** (Fig. 1) had a greater binding affinity than the parent molecule. LUDI has also been used to select fragments which were able to bind to the ATP binding region of DNA gyrase and several of the hit compounds were elaborated to give strongly inhibiting lead compounds [46].

4. Virtual high throughput screening (vHTS)

In virtual screening, rapid docking algorithms are used to search databases of commercially available compounds in order to identify novel molecules predicted to bind to the chosen protein target. Recent advances in technology have made the docking of very large collections of small molecules into a desired molecular target a very rapid and time efficient process. Libraries which are available for screening include the ZINC library [47], Chemnavigator library



Scheme 2. The modular approach to *de novo* design used by SPROUT.

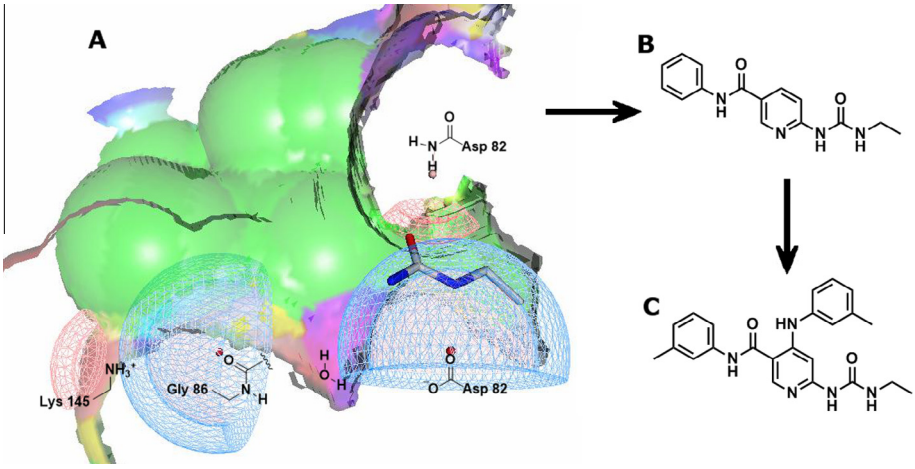


Fig. 2. Design of inhibitors using SPROUT. (A) Four key interactions were chosen in SPROUT and a urea fragment chosen to satisfy two of them, blue targets represent hydrogen, blue targets represent hydrogen acceptor regions, green represents hydrophobic regions; (B) an initial hit compound designed by the program; and (C) a lead compound was developed following SAR.

[48] and the ChemBridge compound library [49]. The identified putative inhibitors predicted using vHTS can be used to obtain a highly focussed library of compounds to be assayed against the protein target. Libraries generated using vHTS have been shown to give hit rates of ~20–30% which is much higher than the hit rates of random HTS which give a hit rate of less than 1% [50]. Some of the most prominent docking software currently in use for virtual screening are AutoDock [51,52], Glide (Schrödinger) [53,54], Gold [55], Dock [56], FRED [57,58] and eHiTS [59,60]. A selection of these programs is discussed below in the context of their recent use in antibacterial drug discovery. In a typical vHTS screen, a library of >10,000 compounds is docked using one of the docking programs. Very large libraries such as the ZINC data base may be pre-screened using a program such as Pipeline Pilot [61] to remove undesirable molecules such as those which violate Lipinski's rule of 5 [62] to reduce the computational time required to screen. From the results generated by the scoring function within the docking program, a 'top-slice' of compounds will be visually inspected to monitor synthetic accessibility, etc. From these, a small library of compounds will be synthesised, or purchased and screened against the enzyme. If an active compound is identified, a SAR study can then be conducted around this molecule. Fig. 3 shows a typical breakdown of a vHTS screening approach.

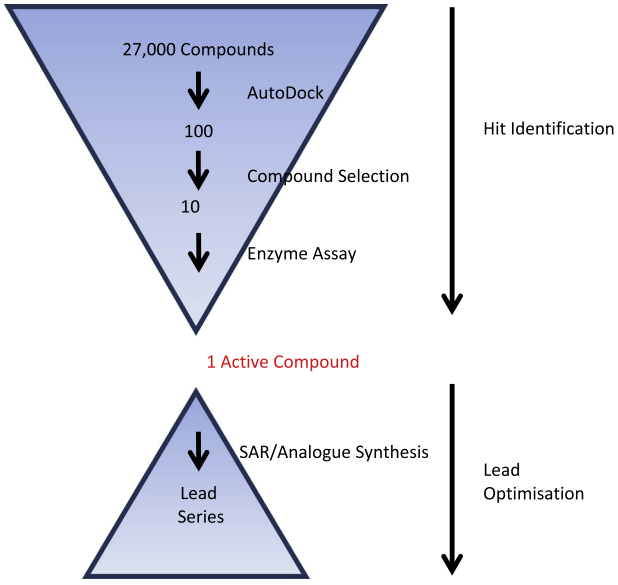


Fig. 3. vHTS identification of active compound based upon screening the Peakdale Molecular Library [63].

4.1. AutoDock [51,52]

AutoDock is a suite of automated docking tools designed to predict how small molecules bind to a receptor of known three-dimensional structure. AutoDock consists of two main programs: 'autodock' which carries out the docking of the desired ligand to a set of grids describing the protein, and 'autogrid' which pre-calculates these grids. In 'autodock' the ligand explores six spatial degrees of freedom, rotation and translation, and an arbitrary number of torsional degrees of freedom within the grid. After docking, AutoDockTools can be used to visualise and analyse the data.

Autodock 4.2 uses the AMBER force field [64] as well as a free energy scoring function based on a linear-regression analysis and a diverse set of protein–ligand complexes with known inhibition constants.

AutoDock 4.2 uses a Lamarckian genetic algorithm [51] to generate a range of docking poses that can be clustered together based on energetic similarity. A number of studies have shown that, rather than the lowest energy cluster, the most populated cluster of the docked ligand conformations is the better predictor of the native state [65].

4.1.1. Identification of inhibitors of New Delhi metallo-β-lactamase 1. (NDM-1) using AutoDock

In recent work at Leeds, AutoDock 4.2 was employed to identify inhibitors of New Delhi metallo-β-lactamase (NDM-1) a metallo-β-lactamase (MBL) which is capable of inactivating all β-lactam antibiotics except azteonam. The first case of an NDM-1 producer was reported in 2008 in a Swedish patient who had previously been hospitalised in New Delhi [66]. NDM-1 is not restricted to a single strain of bacteria and has been identified in a number of unrelated species. No inhibitors have been previously reported of the NDM-1 enzyme. The Peakdale molecular compound library [63] (~27,000 compounds) was docked into the active site of NDM-1 (PDB ID: 3Q6X) using AutoDock 4.2 running in parallel operation mode. The best-scoring 50 compounds, based upon the AutoDock scoring function, were re-scored using the SPROUT scoring function to see which compounds gave comparative results

using both scoring functions to identify strong binding molecules. The top ten scoring compounds were purchased from Peakdale Molecular and the compounds were screened using a nitrocefin florescence assay [67]. Three compounds were observed to inhibit at 100 μM against NDM-1. The best inhibitor, compound **10** (Fig. 4), showed an IC_{50} in the range of 50–100 μM. Current synthetic efforts are focussed upon the design of a library of analogues with improved binding affinity.

4.2. eHiTS [59,60]

eHiTS [59,60] takes a unique approach to docking, by having an innovative algorithm and novel scoring function. The approach breaks ligands into rigid fragments and connecting flexible chains. Each rigid fragment is docked independently and exhaustively within the receptor. A post-match algorithm finds all the matching solutions and reconstructs the original molecule (Scheme 3). The solutions generated are automatically scored by the eHiTS scoring function. This scoring function is based upon the interactions made between surface points on the receptor and ligand. Complementary surface points give a positive score whilst repulsive points give negative scores. The scoring function takes into account components including hydrogen bonding, hydrophobicity, steric clash and entropy lost due to 'frozen' rotatable bonds.

4.2.1. Discovery of inhibitors for staphylococcal pyruvate kinase

eHiTS has been used in the discovery of inhibitors for staphylococcal pyruvate kinase. Work in the Cherkasov group has focussed on discovery of methicillin-resistant *S. aureus* (MRSA) inhibitors [68]. MRSA infections now represent the majority of hospital-acquired infections, which are beginning to penetrate into the community at a large scale and result in an annual death toll in the United States that exceeds that of AIDS [69]. An in-house collection of 255 chemically diverse compounds that were selected from the ZINC library using "antibiotic like" criteria [70] were screened in a MRSA pyruvate kinase enzymatic assay and docked using eHiTS (PDB ID: 3TOT). The molecular docking used eHiTS operating with the standard parameters. For all studied compounds, the docking poses generated by eHiTS reproduced binding modes of the synthetic derivatives obtained by simple replacement of IS-130 **11** (Fig. 1) in the crystal structure. Molecular docking using eHiTS was also used to propose possible structural modification which could be conducted to optimise the IS-130 structure.

4.3. Glide [53,54]

Glide is part of the Maestro modelling package. It approximates a complete systematic search of the conformational, orientation, and positional space of the docked ligand. In this search, an initial rough positioning and scoring phase that dramatically narrows the search space is followed by torsionally flexible energy optimisation on an OPLS-AA [71] non-bonded potential grid for a few hundred surviving candidate poses. The very best candidates are further refined via Monte Carlo sampling of pose conformation; in some cases, this is crucial to obtaining an accurate docked pose. Selection of the best pose uses a model energy function that combines empirical and force-field based terms.

4.3.1. Inhibitors of Francisella tularensis FabI identified by Glide [72]

Glide has been used in the design inhibitors of *Francisella tularensis*. This facultative intracellular Gram-negative bacterium is responsible for the disease tularemia. Without treatment, the mortality rate can be as high as 5–15% for type A strains and 30–60% for the severe systemic and pneumonic forms of the disease. The Legler group has worked on identifying potential inhibitors against *F. tularensis* FabI. After an initial similarity search of the ChemNavigator database (14 million compounds) to known inhibitors using Pipeline Pilot, the top 2000 results and 1150 approved drugs from the DrugBank [73] were subjected to a Glide screening protocol docking using the native crystal structure of *F. tularensis* FabI (PDB ID: 3NRC). The top 200 scoring compounds were visually inspected and 65 commercially available compounds were selected for experimental evaluation. Seven hits were identified using a *F. tularensis* enzymatic assay with IC_{50} values of 5–50 μM using this approach including **12** (Fig. 1) which had an IC_{50} of 6 μM against FabI.

4.4. OEDocking FRED [57,58]

FRED – Fast Rigid Exhaustive Docking, designed and marketed by OpenEye, docks molecules using an exhaustive search algorithm that systematically searches rotations and translations of each conformer of the docked ligand within the active site at a specified resolution. FRED screens the compounds by finding energetically favourable interactions between the protein and the ligand. During an exhaustive search, unrealistic binding poses are filtered, and those that survive are scored. Following this search, the 100 top scoring poses are subject to systematic solid body optimisation (a local exhaustive search at a finer resolution than the global exhaustive search). The best scoring pose is then used to rank the ligand against other ligands in the screening database. The protein is held rigid during the docking process, as are the conformers of the ligand. Ligand flexibility, however, is implicitly included by docking a conformer ensemble of each molecule.

4.4.1. Mimics of the T7-loop of FtsZ [74]

Inhibition of cell division by the SOS response protects bacterial organisms against antibiotics that target cell wall synthesis including the β-lactam antibiotics. SulA inhibits the polymerisation of FtsZ monomers, an essential process leading to bacterial cell division by binding to the T7-loop of FtsZ. Recently the group of Shaw have worked on mimicking the T-7 loop of FtsZ. The crystal structure of FtsZ complexed to SulA from *Pseudomonas aeruginosa* PDB

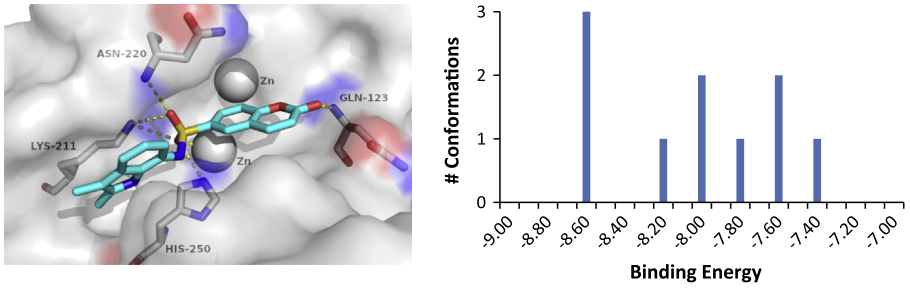
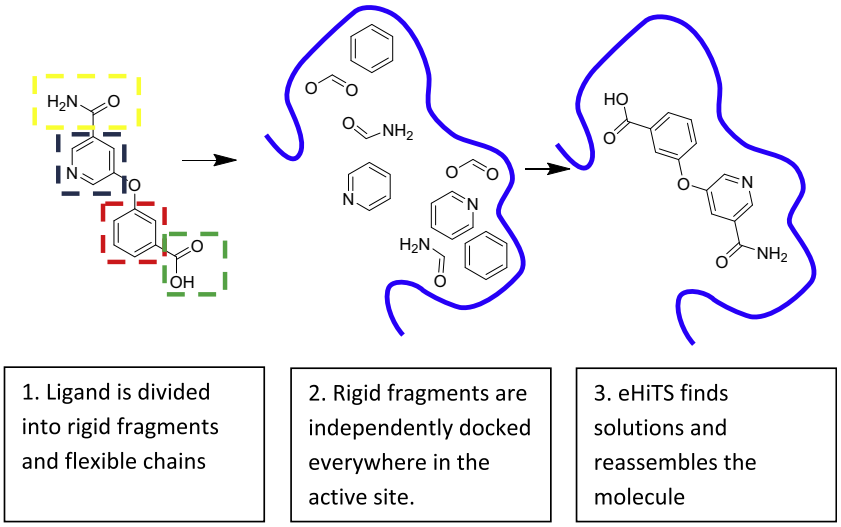


Fig. 4. (a) Compound **9** identified by vHTS of the Peakdale commercial library using AutoDock docked into NDM-1 (3Q6X) showing key binding residues and (b) AutoDock clustering of results.



Scheme 3. eHiTS workflow diagram.

ID: 1OFU was used for docking studies. From visual inspection, it was found that the T-7 loop of FtsZ is the main point of contact between FtsZ and SulA. A library of compounds which were complimentary to the SulA protein were first minimised using OMEGA [75] and then docked into the crystal structure using FRED. Two core molecules, including **13** (Fig. 1), were identified and further runs were conducted optimising the interactions with the receptor which lead to the identification of biologically active compounds.

5. Summary

Structure based drug design provides an excellent platform for the identification of novel antibacterial agents. Inhibitors of bacterial proteins have been identified using *de novo* design software such as SPROUT and identified by virtual screening of compound databases using software such as AutoDock. Structure-based drug design can also be used to find modifications to existing compounds to improve their binding. As computing power continues to improve and the number of bacterial targets for which crystal structures are available increases, structure-based drug design will become an increasingly prominent tool for antibacterial drug discovery.

Acknowledgments

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Appendix A. Supplementary material

More details about all the software tools mentioned can be found in the supplementary information. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2014.05.008.

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