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Structure-Based Drug Design to Overcome Drug Resistance: Challenges and Opportunities

Rafaela S. Ferreira¹ and Adriano D. Andricopulo²,*

¹Biochemistry and Immunology Department, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Belo Horizonte, Minas Gerais, Brazil-CEP 31270-901; ²Laboratório de Química Medicinal e Computacional, Instituto de Física de São Carlos, Universidade de São Paulo, Av. Trabalhador São-carlense, 400, São Carlos - São Paulo - Brasil - CEP 13566-590

Abstract: Drug resistance is a common concern for the development of novel antiviral, antimicrobial and anticancer therapies. To overcome this problem, several strategies have been developed, many of which involving the theme of this review, the use of structure-based drug design (SBDD) approaches. These include the successful design of new compounds that target resistant mutant proteins, as well as the development of drugs that target multiple proteins involved in specific biochemical pathways. Finally, drug resistance can also be considered in the early stages of drug discovery, through the use of strategies to delay the development of resistance. The purpose of this brief review is to underline the usefulness of SBDD approaches based on case studies, highlighting present challenges and opportunities in drug design.

Keywords: Drug discovery, drug resistance, structure-based drug design, therapeutic agents.

INTRODUCTION

Drug discovery is a complex, costly and time-consuming process. In addition to the usual hurdles faced in the design of small molecule drug candidates, the development of antimicrobial, antiviral and anticancer agents involves an additional challenge: the rapid rise of drug resistance [1, 2]. At the molecular level, several mechanisms have been associated with resistance. Given that the targets of these therapies are quickly proliferating cells, there is a high likelihood that mutations will occur. Another possibility is the upregulation of the target, making it necessary to increase drug concentrations to maintain efficacy. In addition, often drugs can be inactivated by chemical modifications, such as oxidation, chemical hydrolysis and conjugation. The drug concentration at the site of action can also be reduced due to efflux pumps [3-6].

This problem is aggravated by the fact that while resistance quickly develops after a drug enters the clinic, developing a new drug is a much harder and slower process. Furthermore, very few medicines have been recently discovered in some areas in which drug resistance is common, such as antimicrobial therapy [1]. It is therefore of surmount importance to understand drug resistance mechanisms and to develop drug design strategies which can rescue the activity of known drugs or that are robust to target mutations [2, 6, 7]. In this brief review, we highlight structure-based drug design (SBDD) approaches that are currently applied to overcome and avoid resistance to drug therapy.

The use of SBDD approaches has a long and rich history in drug discovery [8-13]. Structural information about the molecular target can aid either the discovery of new inhibitors through virtual screening [14-16], or the optimization of known ligands [11, 17]. Herein we present case studies that illustrate important strategies in which SBDD play an essential role to overcome drug resistance. By knowing the structure of mutant proteins, crucial differences between the wild type protein and the resistance mutant can be exploited, so that the mutants can be directly targeted. Additionally, inhibitors can be designed to avoid or at least delay the rise of resistance, for example by exploiting interactions which are essential for interaction with the substrate, in such way that the rise of resistance would cost lost in function and therefore become unlikely. There are also strategies that, instead of focusing on the original target of the drug, focus on targeting other resistance mechanisms, i.e. efflux pumps or enzymes that chemically inactivate the drug. Such strategies yield drugs for combination therapies (Fig. 1). In addition to presenting the strategies above, we discuss some of the challenges and perspectives in the field.

TARGETING MUTATED PROTEINS

The development of small-molecule modulators that target mutated proteins is probably the most straightforward SBDD approach to overcome drug resistance. In cases where structures of resistant mutants are known, new drugs can be designed to bind not only to the wild-type target, but also to the mutants. Several examples have been described for this commonly employed strategy, in anticancer [18], antiviral [19] and antimicrobial therapy [20, 21]. A recent and interesting example was the development of new kinase inhibitors to overcome imatinib resistance.

The development of imatinib represents a major advance for the treatment of chronic myeloid leukemia (CML). This drug targets the ATP binding site in Abl, inhibiting this kinase by binding to its inactive conformation and stabilizing it. In spite of its efficacy, clinical resistance has been observed soon after its release. In the first reports, a now widely known mutation of the gatekeeper residue Thr315 to an Ile was detected [22]. In this case, knowledge of the crystal structure of the complex provided an explanation for resistance: the side chain of Thr315 makes a hydrogen bond to imatinib, while in the Ile mutant this interaction is lost (Fig. 2) [23]. This discovery was followed by other findings of resistant clinical isolates containing several different mutations, which either change the dynamic of the kinase flexibility, impairing its ability to adopt the inactive conformation to which imatinib binds, or disrupt protein-drug interactions [5, 24]. The importance of this clinical issue motivated the development of a second generation of inhibitors [24]. Based on crystal structures of complexes between imatinib (and other kinase inhibitors) and the wild-type and mutant Bcr-Abl [23, 25], the use of SBDD approaches led to the development of nilotinib, an inhibitor 10 to 50-fold more potent than the original drug [18]. Nilotinib has proven to be efficacious against most of the
imatinib-resistant CML isolates, except for those containing the T315I gatekeeper mutation (for which most of the available drugs do not work) [26, 27]. In addition to the improved profile against mutants, recent clinical trials also revealed the potential of the drug for long-term CML treatment compared to imatinib [28, 29].

Even with the success of nilotinib, the high frequency of the T315I mutation encouraged the development of new agents against this protein. As observed in the case of nilotinib, several other compounds were active against all mutant strains, except T315I. To overcome the resistance caused by the T315I mutation, successful approaches included the evaluation of inhibitors of other tyrosine kinases which either exploit other binding sites of the enzyme or bind in a different region of the (imatinib) ATP binding site.

The Aurora kinase inhibitor tozarsetib (MK-0457, VX-680) was active against this mutant strain and in CML patients bearing this mutation, presenting a tolerable safety profile [30-32]. The crystal structure of this compound bound to Bcr-Abl allowed the understanding of the intermolecular interactions and activity against the T315I mutant. While in the case of imatinib the interaction with Thr315 allows one of two essential hydrogen bonds between protein and ligand, molecular modeling studies of tozarsetib suggested that it would form only a weak hydrogen bond to this residue, while forming other five hydrogen-bonds to the protein (Fig. 1). In addition, this inhibitor binds to the enzyme active conformation [31].

The structural studies were useful for the understanding of the main binding requirements, and therefore, for further structure-based drug design efforts towards the identification of ligands having improved affinity and potency [23, 33].

Another strategy to overcome imatinib resistance consists in combining ATP binding site inhibitors with allosteric inhibitors. The first compounds that act through this mechanism (GNF-2 and GNF-5) were identified by experimental screening [34]. Later, structural studies revealed that they bind in the myristate binding site. These compounds were able to overcome resistance of several imatinib-resistant CML cells, and when combined with nilotinib were also effective against the T315I mutation. Moreover, it was shown that resistance is less likely to arise if a combination of GNF-2 and imatinib is used. In addition to showing the clinical potential of this class of compounds, especially when combined with ATP-competitive inhibitors, this study revealed the binding mode of the compounds, providing a molecular basis for further SBDD efforts [35]. Further SAR investigations led to the development of a new class of nanomolar allosteric inhibitors of Bcr-Abl [36]. Together, these studies foster the design of drugs that act through this mechanism.

**DRUGS WITH A DUAL MECHANISM OF INHIBITION**

A promising strategy to overcome or avoid the rise of drug resistance is to identify compounds that bind to more than one target. This approach has proven useful for the development of drugs for imatinib-resistant CML strains, through the design of dual inhibitors of Bcr-Abl and Src kinases. Src kinases are activated by Bcr-Abl and involved in multiple oncogenic pathways. Therefore, inhibition of both steps of the pathway should provide a beneficial effect. Given the similarity between these kinases, it is viable to design inhibitors effective against both, such as the drug dasatinib, a picomolar ATP-competitive inhibitor of both enzymes. The crystal structure of this inhibitor in complex with Bcr-Abl has been reported, whereas the molecular docking of the compound against Src suggests a similar binding mode [7, 37]. As observed with nilotinib, dasatinib was active against most imatinib-resistance strains, except to T315I [38]. The crystal structure of dasatinib bound to the Abl kinase domain revealed that the inhibitor binds to multiple conformational states of this kinase, while imatinib requires the inactive conformation [39].

Other examples of dual Abl/Src kinase inhibitors are the compounds bosutinib (SKI-606) and bafetinib (INNO-406, NS-187). Bosutinib is effective against CML cells in animal models [40], and in phase II clinical trials in patients with imatinib resistance CML with all Bcr-Abl mutations, except T315I [41]. The development of bafetinib provided another classic example of SBDD. Examination of the Abl-imatinib crystal structure revealed a hydrophobic pocket close to the region where imatinib binds, which was explored leading to the design of bafetinib. This compound is a specific inhibitor of Abl and Lyn kinases, and is effective against 12 of the 13 mutation strains evaluated, but T315I [42-44].

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**Fig. (1).** SBDD strategies to overcome or avoid drug resistance.
TARGETING SPECIFIC PROTEINS THAT LEAD TO 
DRUG RESISTANCE

In many cases, resistance emerges due to other mechanisms of resistance (not related to protein mutations) that turn the original drug ineffective. Thus, targeting the resistance mechanism allows the development of new medicines to be used in combination to the original drug. SBDD approaches are also useful in these cases. A classic example is the development of β-lactamase inhibitors [6]. β-lactamase is known as the main cause of bacterial resistance to penicillins, due to its ability to hydrolyze the β-lactam ring, leading to the inactivation of the drugs. There are several classes of β-lactamases (A, B, C and D) organized according to their structural and mechanistic similarities. To overcome resistance, there have been intense efforts to develop either penicillins that cannot be cleaved by these enzymes, or β-lactamase inhibitors. In the latter case, even though the antimicrobial activity of these inhibitors is low, they are able to rescue the efficacy of other drugs. Clavulenate, sulbactam and tazobactam, used in combination with penicillins, are examples of drugs which act through this mechanism. While clavulanic acid was isolated from Streptomyces clavuligerus in the 1970s, sulbactam and tazobactam were developed as analogues. An important limitation of these compounds is the limited spectrum, especially for their lack of efficacy against class C β-lactamases.

Throughout three decades of development of β-lactamase inhibitors, hundreds of crystal structures (in the apo form or bound to different inhibitors) made possible a deep understanding of the mechanisms of inhibition involved [6]. Several SBDD efforts were made to allow the discovery of potent inhibitors (lactams and non-lactams, Fig. 3), including the use of virtual screening of compound libraries [15, 45, 46] and structure-guided optimization [47-51].

The development of bridged monobactams and sulfactams is a good example of the application of structure-based drug design. An analysis of the crystal structure of Citrobacter freundii 1203 β-lactamase in complex with aztreonam suggested the importance of a conformational change in the inhibitor to allow the hydrolysis of the acyl-enzyme intermediate. Based on that, bridged compounds were designed aiming to hinder the conformational change necessary for hydrolysis, leading to the discovery of a class C β-lactamase inhibitors with very low hydrolysis rates. In addition, these compounds were able to reduce up to 200-fold the minimum inhibitory concentration (MIC) of ceftiraxone [52]. Further studies led to a bridged sulfactam, which was an effective inhibitor against both class A and C β-lactamase [55]. Similarly, trycyclic carboxenams were designed to displace a water molecule involved in the deacylation step, preventing antimicrobial hydrolysis and resulting in class C β-lactamase inhibitors [53, 56].

A concern with β-lactam inhibitors is that they frequently upregulate β-lactamase, which in turn affects their efficacy. Therefore, there is a high interest in the development of non β-lactams inhibitors. To this end, SBDD efforts led to the identification of boronic acids as tetrahedral reaction analogues. Several AmpC β-lactamase inhibitors with Ki in the low nanomolar range have been reported, while crystal structures of enzyme-inhibitor complexes have provided valuable insights for drug design. Furthermore, the inhibitors were shown to decrease MIC of penicillins, without upregulation of β-lactamase [47-50, 57-59]. Several other chemical classes were discovered by virtual screening and later optimized by SBDD, leading to low micromolar β-lactamase inhibitors that do not upregulate β-lactamase expression [54, 60]. Virtual screening studies also resulted in the identification of phthalimide inhibitors [61] and several families of fragments that inhibit AmpC [15] or CTX-M β-lactamase [46], providing compounds for lead discovery and optimization.

Aminoglycosides are antimicrobials that lose efficacy due to chemical modifications, such as phosphorylation, acetylation and adenylylation [62]. Several of the enzymes that chemically modify these drugs are known, and can be potential targets for drug development. Some acetyltransferases can also inactivate other classes of antimicrobials, such as quinolones, increasing their clinical relevance [4, 63]. Among the aminoglycoside modifying enzymes, aminoglycoside transferase type III (APH3'-III3a) is a common resistance factor, widely studied and responsible for the phosphorylation of several antibiotics in the class [64]. The determination of the crystal structure of this kinase revealed strong similarities to eukariotic protein kinases [65], motivating the evaluation of inhibitors of human kinases against this enzyme and APH(2''), which confers resistance to most aminoglycosides, leading to the discovery of inhibitors [66]. Later, structures of complexes of one of these inhibitors with APH3'-III3a revealed differences to the binding mode observed against the eukariotic protein kinases, suggesting that selectivity against the aminoglycoside transferases can be achieved [67]. In addition, recent structural studies of complexes between APH(2'') and aminoglycosides provided insights into drug modifications which would be poorly tolerated by the enzyme, being helpful in the design of the next generation of aminoglycosides [68]. Structures of other aminoglycoside phosphotransferases, acetyltransferases and adenylyltransferases have also been determined, providing a more solid basis for SBDD against these enzymes [69-72].

Another common mechanism of antimicrobial resistance is the reduction of drug concentrations in the bacteria due to efflux pumps, some of which are highly promiscuous and can cause multidrug resistance (MDR) [3, 73-75]. Therefore, inhibiting the activity of these pumps can potentially rescue the activity of multiple drugs, as exemplified by compounds that rescue activity of antitumor [76-78] and antimicrobial drugs. These perspectives motivated studies to understand the MDR efflux pump mechanism, and to discover
binding sites for drug development. An example is the determination of crystal structures of the AcrB pump in the presence or absence of substrates, which allowed the identification of a binding site [79, 80]. Later, complexes with other antimicrobials demonstrated the existence of another binding pocket, which could accommodate larger ligands. Together, these studies allowed a better understanding of the mechanism of this efflux pump, leading to the characterization of two multidrug binding sites for drug discovery [81].

**ESSENTIAL SUBSTRATE INTERACTIONS TO AVOID THE DEVELOPMENT OF RESISTANCE**

An important limitation of the approaches described so far is that they are frequently a step behind. First, resistance has to arise, next, the mechanism involved has to be understood, and then, new agents can be developed. Consequently, uncovering new mechanisms of drug resistance is only the first step in the long process of developing more effective drugs. A new approach involving the design of drugs for which resistance is less likely to arise would change the current scenario. To this end, it has been successfully described the design of inhibitors that interact with regions of the protein less likely to mutate, such as residues which are essential for the function of the target. The development of HIV protease inhibitors illustrates two of such approaches: the design of compounds based on the substrate envelope hypothesis or that strongly interact with the protein backbone.

The rationale behind the first approach is straightforward. If the inhibitors occupy a region in the active site that coincides with the consensus occupied by the substrates, drug resistance is less likely to occur, since mutations that lead to drug resistance would be detrimental to the protease activity. This common volume occupied by the protein less likely to mutate, such as residues which are essential for the function of the target. The development of HIV protease inhibitors illustrates two of such approaches: the design of compounds based on the substrate envelope hypothesis or that strongly interact with the protein backbone.

Nano- and picomolar HIV protease inhibitors that satisfy the substrate envelope constraints have been described [84]. Importantly, potent inhibitors that were developed based on this hypothesis indeed showed a flatter activity against a panel of resistance mutants, whereas compounds that protruded the substrate envelope usually were less potent against the mutant proteins [85]. This definition has been recently modified to include protein dynamics [86]. The substrate envelope hypothesis has also been shown to agree with data for a series of HCV protease inhibitors. As observed for the HIV protease inhibitors, compounds for which resistance is more common protrude from the substrate envelope volume [87]. Correlation studies performed for several targets suggest that this concept can be applied in a more general way to design enzyme inhibitors. A retrospective analysis for inhibitors of anticancer, antiviral and antimicrobial enzymes, revealed that resistance is less common for compounds that occupy only the consensus volume of the respective substrate envelope [88].

A similar approach, based on the development of inhibitors which optimize interactions with the HIV protease backbone, led to the development of darunavir, an FDA approved drug for AIDS therapy. Initially, the alignment of several resistant mutant structures revealed that only minimal changes were observed in the protein backbone. Therefore, given that mutations usually do not affect interactions with the protein backbone, the optimization of these interactions would lead to a decreasing of its ability to create resistant mutants. The optimization of the lead compound allowed the development of darunavir, an antiviral with remarkable potency against HIV strains resistant to several other HIV protease inhibitors (Fig. 4) [89]. It is interesting to note that, even though darunavir was not developed based on the substrate envelope hypothesis, it fits the consensus substrate volume [90]. Recent reports demonstrated the development of novel potent antiviral compounds, with a better profile against resistance mutants compared with that of darunavir, which exploited additional hydrogen bonds to the protein backbone [19, 91].
CONCLUSION

The threat of drug resistance is becoming uncomfortably close to reality for a growing number of therapeutic classes, representing a major challenge for the pharmaceutical industry. There is an urgent need for novel treatments aimed at combating the rapid rise in drug resistance. The use of modern SBDD approaches has proven successful in a number of cases to combat drug resistance, as demonstrated by the cases reviewed here. Given the extreme importance of this field and the successes obtained so far, it is expected that structural information will be increasingly more applied in this area. The application of several different strategies has strong relevance, as it will allow us to evaluate which approaches are more successful and prioritize strategies to be employed in different circumstances. It is important to keep in mind that even when a drug is designed to avoid the rise of resistance, it may exhibit a variety of problems. For some of the agents that have been effectively designed to be resistant to mutations, the emergence of new mutants (not sensitive to the drug) may occur, posing serious danger to the treatment. It is important to keep in mind that even when a drug is designed to avoid the rise of resistance, it may exhibit a variety of problems. For some of the agents that have been effectively designed to be resistant to mutations, the emergence of new mutants (not sensitive to the drug) may occur, posing serious danger to the treatment.

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