ELSEVIER

Structure-based methods for predicting target mutation-induced drug resistance and rational drug design to overcome the problem

Ge-Fei Hao[1], Guang-Fu Yang[1] and Chang-Guo Zhan[2]

[1] Key Laboratory of Pesticide & Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, PR China
[2] Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536, USA

Drug resistance has become one of the biggest challenges in drug discovery and/or development and has attracted great research interests worldwide. During the past decade, computational strategies have been developed to predict target mutation-induced drug resistance. Meanwhile, various molecular design strategies, including targeting protein backbone, targeting highly conserved residues and dual/multiple targeting, have been used to design novel inhibitors for combating the drug resistance. In this article we review recent advances in development of computational methods for target mutation-induced drug resistance prediction and strategies for rational design of novel inhibitors that could be effective against the possible drug-resistant mutants of the target.

Introduction

With the development of computer science and structure biology, structure-based drug design has become one of routine approaches of drug discovery today. Aided by structure-based design, many pharmacologists usually focus on improving the potency of drug candidates from micromolar to nanomolar and even picomolar level. However, the stronger the selection pressure, the more rapidly resistance develops because drug target has been proved to be plastic [1]. Take HIV-1 virus as an example, it has been estimated that there are 10^4–10^5 mutations every day for each single residue in an untreated HIV-1 infected individual [2]. Hence, it is an interesting task and urgent demand to develop new strategies to combat drug resistance.

Generally speaking, drug resistance can be divided into several categories (Fig. 1) [3], such as target mutation, epigenetic modifications represented by gene expression variations of the target protein [4] and drug bypass signaling [5,6]. It is desirable to account for the possible drug resistance in the course of drug discovery to overcome the drug resistance as much as possible. Thus, it is interesting to computationally predict the possible target mutation-induced drug resistance and design possible inhibitors that are also effective against the resistant variants (RVs) inhibitors. In this review we focus on recent advances of structure-based approaches to drug resistance prediction and molecular design strategies to combat target mutation-induced resistance.

Corresponding author: Yang, G.-F. (gfyang@mail.ccnu.edu.cn) Zhan, C.-G. (zhan@uky.edu)

Structure-based prediction of mutation-induced drug resistance

The fast and precise prediction of drug resistance mutations could help to avoid therapy failure and/or facilitate therapy redesign after failure. Hence, various computational methods have been used to carry out mutation-induced drug resistance prediction based on the known RV of the target. Commonly, the side chains of amino acids in the target protein structure are replaced by the corresponding ones in a mutant during the computational modeling which aims to model the mutant structure. Molecular docking [7,8], molecular dynamics (MD) simulation [9–13] and computational mutation scanning (CMS) [14,15] methods have been used to determine the binding structures of a drug in various mutants.

Because of the high efficiency of the computation, molecular docking is a good choice for the resistance prediction of a large number of mutants or compounds. For a potential problem, the simple docking cannot deal with the highly flexible protein–inhibitor complex structures very well. MD simulation has an advantage in conformational flexibility sampling and researchers can peer into the motion at atom level from the obtained MD

1359-6446/06/$ - see front matter http://dx.doi.org/10.1016/j.drudis.2012.06.018

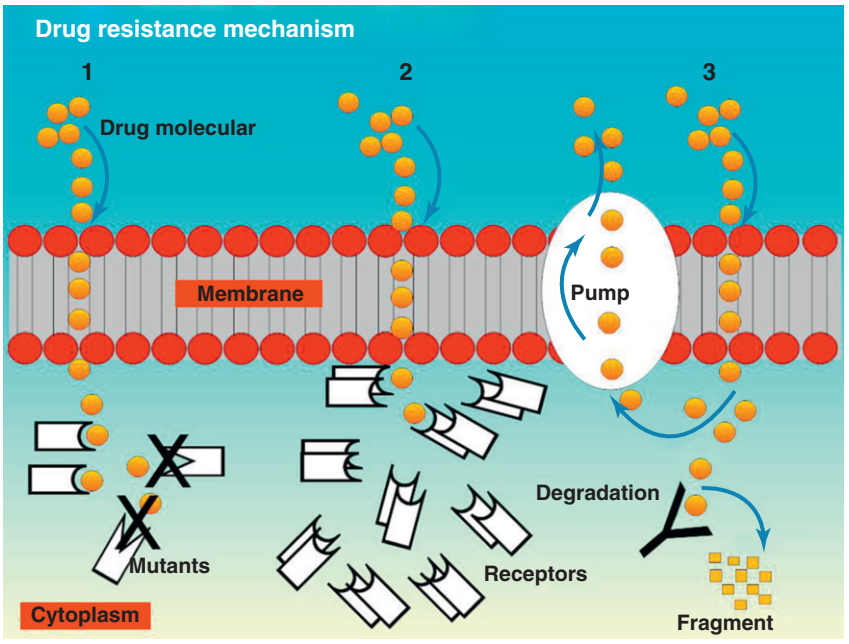

FIGURE 1

Drug resistance mechanisms. Drug resistance can be divided into two main categories: target mutation and non-target mutation. Target mutation affects the binding of the inhibitor (1). Non-target mutations include epigenetic modifications (2) and drug bypass signaling (3). Epigenetic modifications can be grouped into DNA methylation, histone modifications and nucleosome positioning. A representative is gene expression variations of the target protein (2). Resistance can also occur on drug bypass signaling pathway, which results in less drug accumulation by increasing drug elimination or metabolizing (3).

trajectories. On the down side, the MD method is relatively more time-consuming and might not be suitable for simulating a large number of mutants.

To predict mutation-induced shift of the binding free energy for a given mutant, Hao *et al.* [15] developed a novel drug resistance prediction method – CMS, which is a reasonable combination of the MD simulation on wild-type (WT) protein target and subsequent mutational scanning (one-step perturbation) for a larger number of mutants. For the possible limitation of the methodology, the CMS method can be accurate only under a presumption

TABLE 1

Computational drug resistance prediction capability

Year	Method	Target	Reported prediction accuracy[a]				Refs
			Criterion 1	Criterion 2	Criterion 3	Criterion 4	
Phenotypic predictions							
2008	Molecular dynamics	HIV protease				<1.5 kcal/mol	[12]
2008	Molecular modeling protocols	HIV protease			$R^2 = 0.61$		[8]
2008	Vitality value calculation	HIV protease				<1.2 kcal/mol	[43]
2008	Molecular dynamics with free energy/variability value	HIV protease	88%				[44]
2009	Molecular dynamics	Epidermal growth factor receptor (EGFR)			$R^2 = 0.84$		[45]
2009	Molecular dynamics	ACCase			$R^2 = 0.74$		[46]
2009	Molecular dynamics	PPO			$R^2 = 0.84$		[13]
2009	Proteochemometric modeling	HIV protease			$R^2 = 0.92$		[47]
2009	Molecular interaction energy components and support vector machine	HIV protease		86–93%	$R^2 = 0.81$–0.92		[17]
2010	Computational mutation scanning	HIV protease	96%	82%	$R^2 = 0.75$		[15]
Genotypic predictions							
2006	Decision trees, neural networks, support vector regression, least-squares regression and least angle regression	HIV protease and reverse transcriptase		80.1%			[48]
2006	Recurrent neural networks	HIV protease	81.4–94.7%				[49]
2007	Item set boosting	HIV reverse transcriptase			$R^2 = 0.55$–0.94		[50]
2008	Support vector machine, the radial basis function network, and *k*-nearest neighbor	HIV protease and reverse transcriptase	88.0%				[51]

TABLE 1 (*Continued*)

Year	Method	Target	Reported prediction accuracy[a]				Refs
			Criterion 1	Criterion 2	Criterion 3	Criterion 4	
2009	Artificial neural network, random forest, and support vector machine committee	HIV reverse transcriptase			R^2 = 0.73		[52]
The consensus predictions							
2008	Multivariate statistical procedures	HIV reverse transcriptase and protease	65–80%				[53]
2008	Fitness landscape	HIV protease			R^2 = 0.47–0.84		[54]

[a] Various criteria used to represent the prediction accuracy in the references cited:Criterion 1: Percentage of the correctly predicted resistance and non-resistance mutations.Criterion 2: Percentage of the correctly predicted drug resistance levels (high, middle, low and no resistance) in which the low resistance level means less than tenfold resistance (in terms of the IC_{50} value increase), the middle resistance level means less than 100-fold but higher than tenfold resistance and the high resistance level means higher than 100-fold resistance.Criterion 3: Correlation coefficient (R^2) for the linear correction between the computational binding free energy changes and the corresponding experimentally derived binding free energy changes.Criterion 4: Standard deviation of the computational binding free energies from the corresponding experimentally derived binding free energies.

that the mutation does not considerably change the binding mode of the drug. In other words, the CMS calculation could significantly overestimate the drug resistance associated with the mutant when the mutation actually causes a considerable change in the binding mode. Based on the CMS calculations, the mutation-induced drug resistance mechanisms include the following categories: (i) decrease in the enthalpy contribution to the binding affinity, (ii) decrease in the entropic contribution to the binding affinity, (iii) decrease in both the enthalpy and entropic contributions, (iv) no significant change in the enthalpy and entropic contribution, (v) decrease in the enthalpy contribution compensated with increase in the entropic contribution and (vi) decrease in the entropic contribution compensated with increase in the enthalpy contribution.

Statistical learning methods [16] have also been used for sequence-based drug resistance predictions. By using these purely empirical approaches, one only needs to know the primary structure (sequence) of the target protein, but a large number of known resistance and non-resistance mutants are required for the model training. Well-trained models could have satisfactory predictive ability which is measured by the prediction accuracy of the resistance level (Table 1).

Furthermore, there have been efforts to develop other effective computational approaches to the drug resistance prediction based on a combined use of structure- and sequence-based methods [17]. In particular, Zhang *et al.* [18] proposed a unique procedure that combines Bayesian statistical modeling with MD simulations to investigate complex interactions of drug resistance mutations of the HIV-1 protease and reverse transcriptase. They presented a statistical procedure that first detects mutation combinations associated with drug resistance and then the molecular basis of their statistical predictions was further studied by carrying out MD simulations and free energy calculations to infer detailed interaction structures of these mutations. Their proof-of-concept study has demonstrated that the insights obtained from the MD simulations guided by the Bayesian inference can shed light on how to improve the potency of drugs to combat the resistance.

Table 1 summarizes the results associated with different methods reported in recent years. Predictive ability of these studies is mainly evaluated by qualitative or quantitative indicators. Qualitatively, drug resistance can be divided into different levels, in which prediction accuracy is in the range of 82–96% for structure-based methods, 80–95% for sequence-based methods and 65–80%

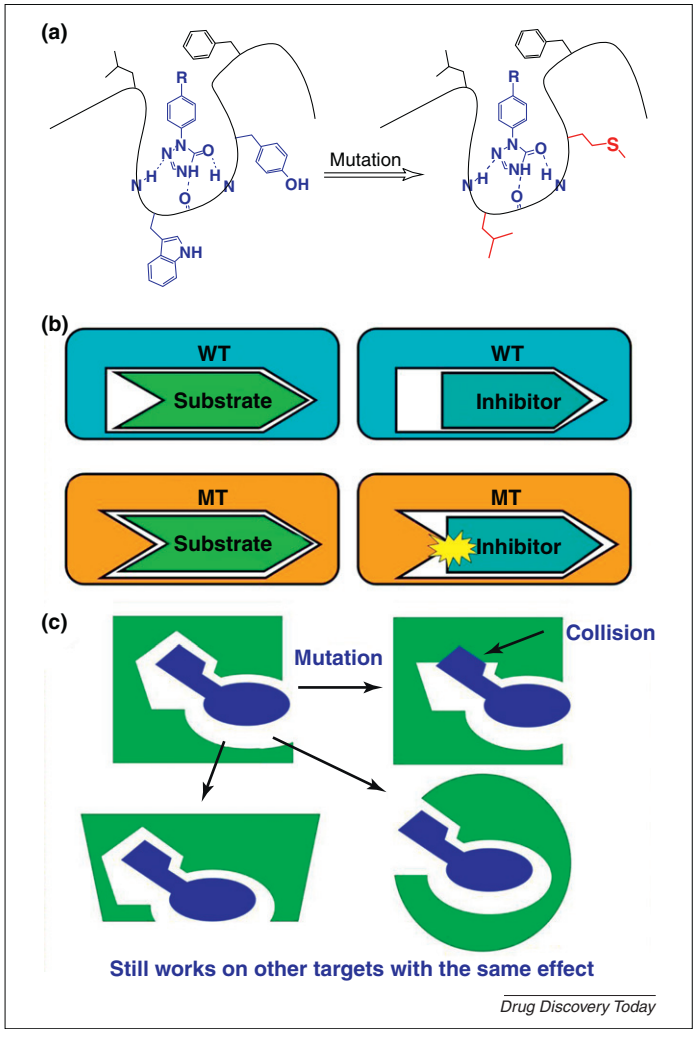

FIGURE 2

Various strategies used to design novel inhibitors for combating the drug resistance. **(a)** Inhibitors designed to have strong hydrogen-bond interactions with the backbone atoms of the target protein can probably reserve important interactions with the mutants and, thus, effectively combat drug resistance. **(b)** Designing inhibitors that have significant interactions mainly with the conserved residues interacted with the substrate can minimize the dependence of the activity on the non-conserved residues. **(c)** Dual/multiple targeting strategy, which uses a single molecular entity to inhibit multiple protein targets, could significantly reduce the likelihood of drug resistance.

for consensus methods. Quantitatively, the predictive ability can also be evaluated by the prediction accuracy or standard deviation of the computational values from the corresponding experimental values. In the quantitative analysis, correlation coefficient (R^2) and the standard deviation between the computational and experimental binding affinities are widely used. Usually, the R^2 value ranges from 0.61 to 0.92 in structure-based methods, 0.55–0.94 in sequence-based methods and 0.47 and 0.90 in consensus methods. The standard deviation of the binding energy calculation is usually under 1.5 kcal/mol.

Structure-based design of RV inhibitors

It is interesting to design and develop more effective drugs that can be active for both the WT protein target and its possible variants. Detailed analysis on a wide range of X-ray crystal structures of protein–drug complexes, along with biological data on drug-resistant mutations, has identified structural factors important for rational design of new inhibitors whose binding affinity with the target is not (or less) affected by the target mutations.

Targeting protein backbone

Amino acid mutations change the side chains of mutated residues, but do not change the backbone. Inhibitors designed to have strong hydrogen-bond interactions with the backbone atoms of the target protein can probably reserve important interactions with the mutants and, thus, effectively combat drug resistance (Figs 2a and 3a) [19–22]. For example, a series of 1-[(2-hydroxyethoxy) methyl]-6-(phenylthio)thymine (HEPT) analogs were computationally designed and synthesized [23] to form two hydrogen bonds with the backbone carbonyl group of Lys101 of HIV-1 reverse transcriptase. Most of these compounds are highly potent inhibitors of WT HIV-1 reverse transcriptase and its resistant mutants.

It is well known that darunavir displayed ultrahigh HIV protease inhibitor potency (K_i = 16 pM) and retained the potency against many highly drug-resistant HIV mutants by forming hydrogen bonds between bis-tetrahydrofuran (bis-THF) moiety and the backbone NH groups of Asp29 and Asp30 [24]. Ghosh *et al.* [25] further predicted that the incorporation of another tetrahydrofuran ring on the bis-THF ligand could provide additional favorable binding with the backbone atoms. The prediction guided them to design and synthesize a series of novel oxatricyclic ligands that 'displayed potent activity against a variety of multidrug-resistant clinical HIV-1 strains, with EC_{50} values ranging from 0.6 to 4.3 nm, a nearly tenfold improvement over darunavir' [25].

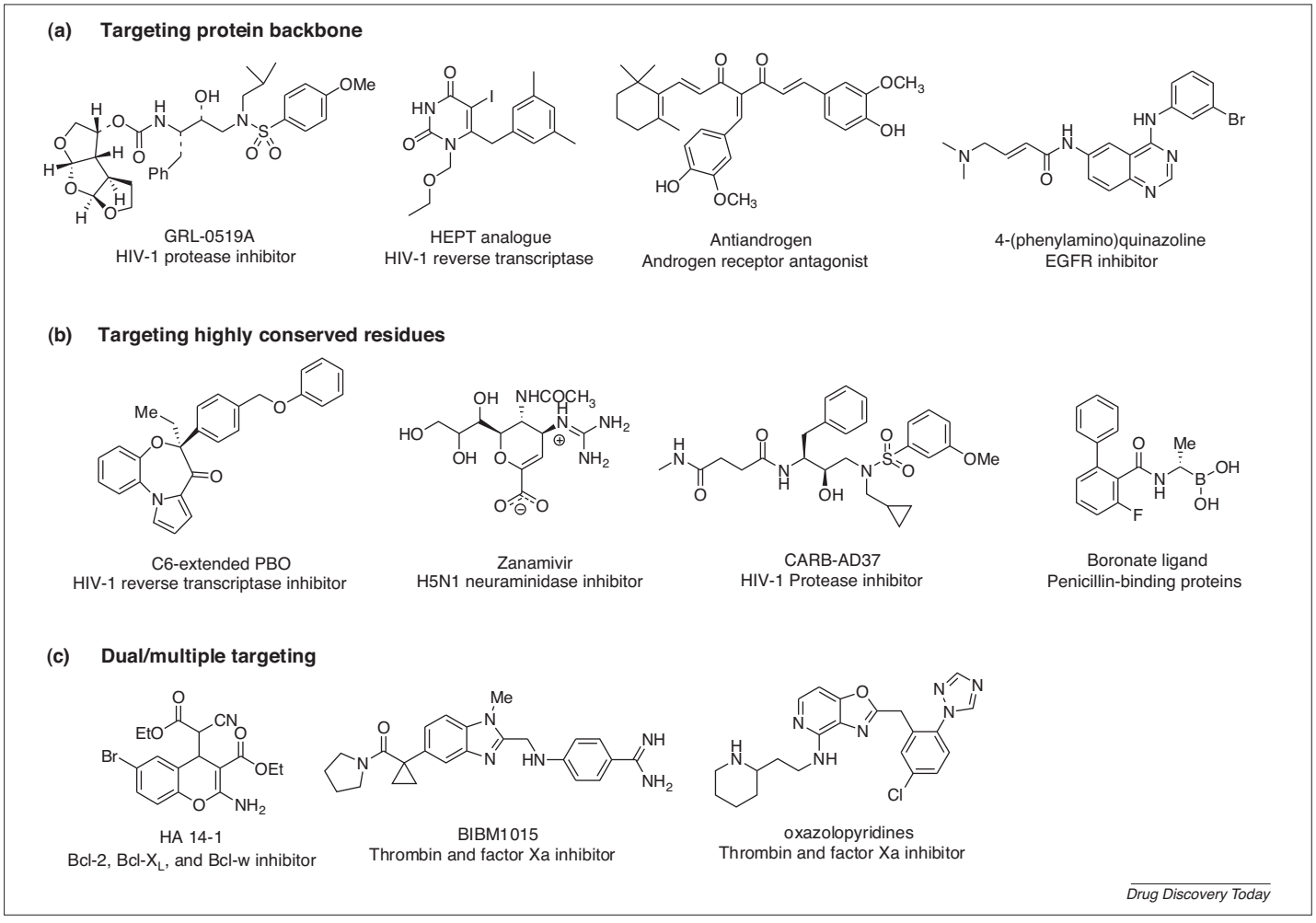

FIGURE 3

Chemical structures of known representative inhibitors (designed by using different strategies) that are potent toward WT and many drug-resistant mutants.

Targeting highly conserved residues

The analysis of the reported resistant mutants demonstrated that few drug-resistant mutations happened on highly conserved residues [26]. Hence, another widely accepted strategy is to design inhibitors that have significant interactions with only the highly conserved residues so as to minimize the dependence of the activity on the non-conserved residues (Figs 2b and 3b) [27–30].

β-Lactam antibiotics have long been used for the treatment of bacterial infections because they bind irreversibly to penicillin-binding proteins (PBPs) that are vital for the cell wall biosynthesis. Many pathogens express drug-insensitive PBPs to render β-lactams ineffective, which reveals a need for new types of PBP inhibitors that are active against the resistant mutants. Contreras-Martel *et al.* [31] identified boronic acid inhibitors that are active against clinically relevant pathogens and may overcome β-lactam resistance by mimicking the tetrahedral catalytic intermediate.

In addition, Schiffer *et al.* developed a 'substrate envelope' hypothesis that inhibitors located within the overlapping consensus volume of the substrates were less likely to be susceptible to drug-resistant mutations than inhibitors that protrude beyond this envelope [32]. As mutations impacting such inhibitors would simultaneously impact the process of substrate perception [33–37]. For an ideal inhibitor located within the substrate envelope, there will be no chance for drug resistance mutation except for the rare coevolution of the protein and substrate [38]. To evaluate this hypothesis, more than 130 new inhibitors of HIV-1 protease were designed and synthesized with and without the substrate-envelope constraints [37]. In general, inhibitors that fit within the substrate envelope have flatter profiles with respect to drug-resistant protease variants than inhibitors that protrude beyond the substrate envelope. Thus, the acquired results from testing this hypothesis are encouraging as they have demonstrated that combining the substrate-envelope hypothesis with structure-based drug design may result in new inhibitors that are less susceptible to drug-resistant mutations.

Dual/multiple targeting

It has been well known that drug combination (combination therapy) is an effective strategy to overcome drug resistance. To improve patient compliance, two or more drugs can also be coformulated into a single tablet. On the down side, complex pharmacokinetic (PK) and/or pharmacodynamic (PD) profiles and unpredictable drug–drug interaction could have a significant impact on the risks and costs of developing multicomponent drugs [39]. Dual/multiple targeting strategy, which uses a single molecular entity to inhibit multiple protein targets, could significantly reduce the likelihood of drug resistance without the extra patient compliance problem (Figs 2c and 3c). For a particular example, *ABCB1* (ATP-binding cassette, sub-family B, *MDR1*) overexpression protects leukemia cells from drug-induced apoptosis and decreases sensitivity of leukemia cells to cytotoxic chemotherapeutic agents. Mutations in *ABCB1* are one of the mechanisms for chemoresistance common to a wide spectrum of cancers. Recent studies showed that myeloid cell leukemia sequence 1 (BCL2-related) gene (*MCL1*) was upregulated in numerous hematological and solid tumor malignancies. Ji *et al.* demonstrated that *MCL1* mediated drug resistance through a different mechanism and the depletion of both *MCL1* and *ABCB1* showed an additive effect in reversing drug resistance and promoting drug-induced apoptosis [40]. So, simultaneous targeting of *MCL1* and *ABCB1* could be an effective approach to overcome drug resistance in leukemia. However, a key challenge of dual/multiple targeting is attaining a balanced activity at each target of interest while simultaneously achieving a wider selectivity and a suitable PK profile.

Concluding remarks

Recent studies have revealed that the efficacy of many small molecule drugs can be hampered by the rapid emergence of drug resistance mutations on the target proteins and that the battle against mutation-induced drug resistance has become increasingly intense [41,42]. Rational strategies to combat mutation-induced drug resistance should be accounted for in the course of drug discovery and/or development to prevent the emergence of resistance as much as possible.

The structure-based methods are particularly useful for computational prediction of resistant mutants and RV inhibitor design. The primary challenge of structure-based drug resistance prediction is how to appropriately balance the prediction accuracy and computational efficiency. It would be an ideal approach to efficiently predict the drug resistance level and understand the resistance mechanism associated with each resistant mutant through an appropriately combined use of structure-based methods and statistical learning methods. Besides, targeting protein backbone, targeting highly conserved residues, and dual/multiple targeting have been recognized as effective strategies for rational design of novel inhibitors with reduced resistance risk. Structure-based drug design could eventually lead to the discovery and development of novel, more potent and safer drugs with potentially different resistance profiles compared to the existing drugs. It would be interesting to further develop and validate novel strategies and/or make an appropriately combined use of available strategies. One can reasonably expect that the use of structure-based methods will become more and more popular in the battle against drug resistance.

Acknowledgements

This work was supported in part by the National Basic Research Program of China (grant no. 2010CB126103), NSFC (grants nos. 20925206 and 20932005), NSF (grant CHE-1111761) and NIH (grant RC1MH088480).

References

1 Schliekelman, P. *et al.* (2001) Natural selection and resistance to HIV. *Nature* 411, 545–546

2 Coffin, J.M. (1995) HIV population dynamics *in vivo*: implications for genetic variation, pathogenesis, and therapy. *Science* 267, 483–489

3 Longley, D.B. and Johnston, P.G. (2005) Molecular mechanisms of drug resistance. *J. Pathol.* 205, 275–292

4 Badia, E. *et al.* (2007) Tamoxifen resistance and epigenetic modifications in breast cancer cell lines. *Curr. Med. Chem.* 14, 3035–3045

5 Qi, J. *et al.* (2011) Multiple mutations and bypass mechanisms can contribute to development of acquired resistance to MET inhibitors. *Cancer Res.* 71, 1081–1091
6 Hu, X. and Xuan, Y. (2008) Bypassing cancer drug resistance by activating multiple death pathways – a proposal from the study of circumventing cancer drug resistance by induction of necroptosis. *Cancer Lett.* 259, 127–137
7 Jenwitheesuk, E. and Samudrala, R. (2005) Prediction of HIV-1 protease inhibitor resistance using a protein-inhibitor flexible docking approach. *Antivir. Ther.* 10, 157–166
8 Pepe, G. *et al.* (2008) Prediction of HIV-1 protease inhibitor resistance by molecular modeling protocols (MMPs) using GenMol software. *Eur. J. Med. Chem.* 43, 2518–2534
9 Layten, M. *et al.* (2006) The open structure of a multi-drug-resistant HIV-1 protease is stabilized by crystal packing contacts. *J. Am. Chem. Soc.* 128, 13360–13361
10 Hou, T. and Yu, R. (2007) Molecular dynamics and free energy studies on the wild-type and double mutant HIV-1 protease complexed with amprenavir and two amprenavir-related inhibitors: mechanism for binding and drug resistance. *J. Med. Chem.* 50, 1177–1188
11 Ode, H. *et al.* (2007) Mechanism of drug resistance due to N88S in CRF01_AE HIV-1 protease, analysed by molecular dynamics simulations. *J. Med. Chem.* 50, 1768–1777
12 Stoica, I. *et al.* (2008) Rapid and accurate prediction of binding free energies for saquinavir-bound HIV-1 proteases. *J. Am. Chem. Soc.* 130, 2639–2648
13 Hao, G.F. *et al.* (2009) Understanding the mechanism of drug resistance due to a codon deletion in protoporphyrinogen oxidase through computational modeling. *J. Phys. Chem. B* 113, 4865–4875
14 Moreira, I.S. *et al.* (2007) Computational alanine scanning mutagenesis – an improved methodological approach. *J. Comput. Chem.* 28, 644–654
15 Hao, G.F. *et al.* (2010) Computational mutation scanning and drug resistance mechanisms of HIV-1 protease inhibitors. *J. Phys. Chem. B* 114, 9663–9676
16 Cao, Z.W. *et al.* (2005) Computer prediction of drug resistance mutations in proteins. *Drug Discov. Today* 10, 521–529
17 Hou, T. *et al.* (2009) Predicting drug resistance of the HIV-1 protease using molecular interaction energy components. *Proteins* 74, 837–846
18 Zhang, J. *et al.* (2010) Detecting and understanding combinatorial mutation patterns responsible for HIV drug resistance. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1321–1326
19 Tie, Y. *et al.* (2004) High resolution crystal structures of HIV-1 protease with a potent non-peptide inhibitor (UIC-94017) active against multi-drug-resistant clinical strains. *J. Mol. Biol.* 338, 341–352
20 Ghosh, A.K. *et al.* (2006) Structure-based design of novel HIV-1 protease inhibitors to combat drug resistance. *J. Med. Chem.* 49, 5252–5261
21 Michalczyk, A. *et al.* (2008) Structural insights into how irreversible inhibitors can overcome drug resistance in EGFR. *Bioorg. Med. Chem.* 16, 3482–3488
22 Ghosh, A.K. *et al.* (2009) Design, synthesis, protein-ligand X-ray structure, and biological evaluation of a series of novel macrocyclic human immunodeficiency virus-1 protease inhibitors to combat drug resistance. *J. Med. Chem.* 52, 7689–7705
23 Wang, X. *et al.* (2012) Design, synthesis, and biological evaluation of 1-[(2-Benzyloxyl/alkoxyl)methyl]-5-halo-6-aryluracils as potent HIV-1 non-nucleoside reverse transcriptase inhibitors with an improved drug resistance profile. *J. Med. Chem.* 55, 2242–2250
24 Lefebvre, E. and Schiffer, C.A. (2008) Resilience to resistance of HIV-1 protease inhibitors: profile of darunavir. *AIDS Rev.* 10, 131–142
25 Ghosh, A.K. *et al.* (2010) Probing multidrug-resistance and protein-ligand interactions with oxatricyclic designed ligands in HIV-1 protease inhibitors. *ChemMedChem* 5, 1850–1854
26 Wang, W. and Kollman, P.A. (2001) Computational study of protein specificity: the molecular basis of HIV-1 protease drug resistance. *Proc. Natl. Acad. Sci. U.S.A.* 98, 14937–14942
27 Fattorusso, C. *et al.* (2005) Specific targeting highly conserved residues in the HIV-1 reverse transcriptase primer grip region. Design, synthesis, and biological evaluation of novel, potent, and broad spectrum NNRTIs with antiviral activity. *J. Med. Chem.* 48, 7153–7165
28 Wang, S.Q. *et al.* (2007) Study of drug resistance of chicken influenza A virus (H5N1) from homology-modeled 3D structures of neuraminidases. *Biochem. Biophys. Res. Commun.* 354, 634–640
29 Butini, S. *et al.* (2009) Specific targeting of highly conserved residues in the HIV-1 reverse transcriptase primer grip region. 2. Stereoselective interaction to overcome the effects of drug resistant mutations. *J. Med. Chem.* 52, 1224–1228
30 Ishima, R. *et al.* (2010) Highly conserved glycine 86 and arginine 87 residues contribute differently to the structure and activity of the mature HIV-1 protease. *Proteins* 78, 1015–1025
31 Contreras-Martel, C. *et al.* (2011) Structure-guided design of cell wall biosynthesis inhibitors that overcome beta-lactam resistance in *Staphylococcus aureus* (MRSA). *ACS Chem. Biol.* 6, 943–951
32 King, N.M. *et al.* (2004) Combating susceptibility to drug resistance: lessons from HIV-1 protease. *Chem. Biol.* 11, 1333–1338
33 Nijhuis, M. *et al.* (2007) A novel substrate-based HIV-1 protease inhibitor drug resistance mechanism. *PLoS Med.* 4, E36
34 Altman, M.D. *et al.* (2008) Computational design and experimental study of tighter binding peptides to an inactivated mutant of HIV-1 protease. *Proteins* 70, 678–694
35 Jorissen, R.N. *et al.* (2009) Additivity in the analysis and design of HIV protease inhibitors. *J. Med. Chem.* 52, 737–754
36 Kairys, V. *et al.* (2009) Toward the design of mutation-resistant enzyme inhibitors: further evaluation of the substrate envelope hypothesis. *Chem. Biol. Drug Des.* 74, 234–245
37 Nalam, M.N. *et al.* (2010) Evaluating the substrate-envelope hypothesis: structural analysis of novel HIV-1 protease inhibitors designed to be robust against drug resistance. *J. Virol.* 84, 5368–5378
38 Kolli, M. *et al.* (2006) Co-evolution of nelfinavir-resistant HIV-1 protease and the p1–p6 substrate. *Virology* 347, 405–409
39 Morphy, R. and Rankovic, Z. (2005) Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* 48, 6523–6543
40 Ji, M. *et al.* (2009) Simultaneous targeting of MCL1 and ABCB1 as a novel strategy to overcome drug resistance in human leukaemia. *Br. J. Haematol.* 145, 648–656
41 de Jong, S. *et al.* (2001) Death receptor ligands, in particular TRAIL, to overcome drug resistance. *Cancer Metastasis Rev.* 20, 51–56
42 Avendano, C. and Menendez, J.C. (2002) Inhibitors of multidrug resistance to antitumor agents (MDR). *Curr. Med. Chem.* 9, 159–193
43 Ishikita, H. and Warshel, A. (2008) Predicting drug-resistant mutations of HIV protease. *Angew. Chem. Int. Ed.* 47, 697–700
44 Hou, T. *et al.* (2008) Evaluating the potency of HIV-1 protease drugs to combat resistance. *Proteins* 71, 1163–1174
45 Balius, T.E. and Rizzo, R.C. (2009) Quantitative prediction of fold resistance for inhibitors of EGFR. *Biochemistry* 48, 8435–8448
46 Zhu, X.L. *et al.* (2009) Computational simulations of the interactions between acetyl-coenzyme-A carboxylase and clodinafop: resistance mechanism due to active and nonactive site mutations. *J. Chem. Inf. Model.* 49, 1936–1943
47 Lapins, M. and Wikberg, J.E. (2009) Proteochemometric modeling of drug resistance over the mutational space for multiple HIV protease variants and multiple protease inhibitors. *J. Chem. Inf. Model.* 49, 1202–1210
48 Rhee, S.Y. *et al.* (2006) Genotypic predictors of human immunodeficiency virus type 1 drug resistance. *Proc. Natl. Acad. Sci. U.S.A.* 103, 17355–17360
49 Bonet, I. *et al.* (2007) Predicting Human Immunodeficiency Virus (HIV) drug resistance using recurrent neural networks. *International Work-Conference on the Interplay Between Natural and Artificial Computation* pp. 234–243
50 Saigo, H. *et al.* (2007) Mining complex genotypic features for predicting HIV-1 drug resistance. *Bioinformatics* 23, 2455–2462
51 Srisawat, A. and Kijsirikul, B. (2008) Combining classifiers for HIV-1 drug resistance prediction. *Protein Pept. Lett.* 15, 435–442
52 Wang, D. *et al.* (2009) A comparison of three computational modelling methods for the prediction of virological response to combination HIV therapy. *Artif. Intell. Med.* 47, 63–74
53 Almerico, A.M. *et al.* (2008) Docking and multivariate methods to explore HIV-1 drug-resistance: a comparative analysis. *J. Comput. Aided Mol. Des.* 22, 287–297
54 Deforche, K. *et al.* (2008) Estimation of an *in vivo* fitness landscape experienced by HIV-1 under drug selective pressure useful for prediction of drug resistance evolution during treatment. *Bioinformatics* 24, 34–41