



The art of suicidal molecular seduction for targeting drug resistance

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ABSTRACT

The development of drug resistance is one of the most significant challenges of the current century in the pharmaceutical industry. Superinfections, cancer chemoresistance, and resistance observed in many non-infectious diseases are nullifying the efforts and monetary supplies, put in the advent of new drug molecules. Millions of people die because of this drug resistance developed gradually through extensive use of the drugs. Inherently, some drugs are less prone to become ineffective by drug resistance than others. Covalent inhibitors bind to their targets via a biologically permanent bound with their cognate receptor and therefore display more potent inhibiting characteristics. Suicide inhibitors or mechanism-based inhibitors are one of the covalent inhibitors, which require a pre-activation step by their targeting enzyme. This step accrues their selectivity and specificity with respect to other covalent inhibitors. After that pre-activation step, they produce an analogue of the transition state of the catalytic enzyme, which is practically incapable of dissociating from the enzyme. Suicide inhibitors, due to their high intrinsic affinity toward the related enzyme, are resistant to many mechanisms involved in the development of drug resistance and can be regarded as one of the enemies of this scientific hurdle. These inhibitors compete even with monoclonal antibodies in terms of their cost-effectiveness and efficacy.

Introduction

Drug Resistance was first brought into attention when certain antibiotics failed to suppress the growth of some bacteria [1], but related mechanisms were then characterized in many other diseases such as hypertension [2], epilepsy [3], autoimmune diseases [4], hypercholesterolemia [5], asthma [6]. Many infectious diseases like malaria [7], acquired immunodeficiency syndrome (AIDS) [8], tuberculosis [9], nosocomial infections with *Pseudomonas aeruginosa* [10] and perhaps the most notable case of drug resistance i.e. cancer are facing this challenge nowadays [11,12].

Moreover, we have introduced several specific terminologies to describe particular types of drug resistance. Pharmacoresistant epilepsy (PRE) is a term coined to describe the failure of sufficient patient response to at least two appropriately chosen antiepileptic drug regimens [13]. Some patients with type 2 diabetes mellitus manifest persistent poorly controlled diabetes mellitus (PPDM) despite standard medications (should not be confused with insulin resistance, which is another concept in the etiology of diabetes mellitus) [14]. These findings suggest that drug resistance encompasses a wide range of diseases and it is

not circumscribed in the realm of pathogenic diseases or cancer.

To date, a number of different mechanisms were demonstrated for drug resistance (Fig. 1).

1. Development or overexpression of enzymes capable of inactivating or metabolizing drug molecules [13]
2. Changing the structure of target macromolecules, which are inefficient in binding drugs [15]
3. Altering the cell's permeability to drugs or creating a barrier via protective sheaths (like biofilms) [16,17]
4. Actively expelling the drug molecules with membrane efflux pumps [18,19]
5. Hyperactivation of DNA repair systems [20]
6. Cessation of apoptosis or overactivation of autophagy [21].

One of the main attractive points about all of these possible mechanisms is the fact that all of these modifications in cell responses are mediated by mutations in some essential proteins. The development of new drugs with potent binding properties to a conserved domain in the structure of those proteins would suffice to halt the drug-resistant

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Fig. 1. The major mechanisms involved in drug resistance.

behavior of the cells, and conceivably, some recent researches are designed to achieve this goal [22–24].

There are two types of inhibition mechanisms employed by ligands for a specific protein. Irreversible inhibition is accompanied by forming a covalent bond between the drug and receptor, and reversible inhibition is maintained by non-covalent intermolecular interactions as hydrogen bonds, hydrophobic, electrostatic and van der Waals interactions [25–27].

Binding of a reversible inhibitor would likely cause that one drug molecule hampers the activity of a target for a certain period of time. Binding of an irreversible inhibitor of a target macromolecule would generate a permanent bond between the drug and the related target molecule. Consequently, irreversible inhibition of enzymes has the advantage of prolonged pharmacokinetics profile, augmented selectivity, and enhanced potency and efficacy over the more well-known reversible inhibitors [28,29]. These extended effects observed in irreversible inhibitors correlate with their increased rate of toxicity (particularly in case of non-selective covalent inhibition of several structurally related targets) and less use in human diseases [30,31].

There has been a lot of efforts in designing covalent inhibitors for a variety of targets. Aromatase (exemestane) [32], β -lactamase (sulbactam) [33], H^+/K^+ ATPase (omeprazole) [34], EGFR and related tyrosine kinases (afatinib, neratinib, ibrutinib) [35], P2Y purinoreceptor 12 (clopidogrel) [36], ornithine decarboxylase (eflornithine) [37], Monoamine Oxidase B or MAO-B (selegiline) [38], GABA Transaminase or GABA-T (vigabatrin) [39], enoyl-ACP reductase (ethionamide) [40], penicillin-binding proteins (ampicillin) [41], gastric and pancreatic lipases (orlistat) [42], aldehyde dehydrogenase (disulfiram) [43], DOPA decarboxylase (carbidopa) [44], 5- α reductase (finasteride) [45], and even DNA (mitomycin C) [46] or RNA (kanamycin) [47] were the subject of covalent inhibition by different ligands. Some drugs also inactivate the P450 cytochrome complex in the liver by forming irreversible covalent bonds. Abiraterone and methoxsalen are among the covalent inhibitors of the CYP17A1 and CYP2A6 respectively [48,49].

Most intriguingly, there are FDA-approved enzymes that produce their therapeutic activity by forming an irreversible covalent linkage with another protein. For instance, α -1 antitrypsin or α -1 proteinase

inhibitor is a protein used in the treatment of emphysema related to α -1 antitrypsin deficiency and acts by irreversibly attaching to elastase and establishing an inactive highly stable enzyme-enzyme complex [50–52].

In some cases of covalent inhibition, the enzyme lends a hand to its own demise. In this procedure, the enzyme catalyzes the first step of a chemical reaction and then become trapped in an intermediate state, bound covalently to an electrophile group in the ligand structure. Hence, the term suicide or suicidal inhibition (or mechanism-based inhibition) refers to this type of inhibition, where the enzyme prepares the ligand to commit suicide [53]. Suicide inhibition is defined by the time-dependent inhibition of enzymes, directed by an active-site mediated covalent drug-enzyme adduct, resulting from catalytic conversion of a substrate-like analogue [54]. The inactivated enzyme by the “seducer” ligand substrate, the “hit”, is often considered to have a “run” effect, consistent with long-lasting time-dependent inhibition of the target. Thus, this type of inhibition has a “hit and run” effect on the biological milieu.

Works made by Smith et al. revealed that the potency of interactions between a ligand and its receptor can be classified in three categories; first, the low-potency affinities that account for decimolar to hundreds of micromolar affinities (for instance, binding of albumin to organic molecules), medium interactions that demonstrate micromolar to nanomolar range affinities (for antibody-antigen complex and many drug-receptors for example), and high affinities, which are observed for enzymes-transitional state complexes, which includes for subpicomolar affinity range [55]. In this respect, suicide inhibitors are even superior to other types of covalent-inhibitors because they exactly mimic the transitional state of the enzymes (but other covalent inhibitors may act on different parts of a protein structure, and not necessarily the active site of an enzyme). Although it might seem exaggerated, suicide inhibitors could be among the most potent inhibitors designed for a biological target.

Hypothesis

An urgent need for strong and potent antimicrobial agents is currently demanded for the prevention and treatment of different diseases, especially superinfections. We should identify the best mechanism for the inhibition of the enzymes activity and go forward to design inhibitors based upon the catalytic activity of the enzymes. Suicide inhibitors are one of the challenging drugs which are hard to design but provide promising opportunities in battling drug resistance. Due to their high potency and selectivity as well as lower propensity for the development of drug resistance, mechanism-based inhibitors can provide a valuable outlook on the problem of drug resistance. There are no current statistical data to prove whether these inhibitors are stronger than previous non-covalent or covalent inhibitors, but they are harder to be designed, identified and synthesized. There are concerns about their prolonged mode of action and possibility of toxicity, but in the case of drug resistance, they provide realistic chances. Suicide inhibitors are particularly invaluable for targeting proteins of bacterial cells where there is a low homology and similarity between the targeting protein in the bacteria and human proteins, and the risk of toxicity is appreciably lower [56].

Evaluating the hypothesis

Drugs with mechanism-based inhibition provide a more appropriate and acceptable pharmaceutical profile, i.e. better pharmacodynamics due to more selective inhibition of their cellular counter-part and better pharmacokinetics because the half-life of inhibition exceeds the plasma concentration of drugs (is dependent on the clearance of the targeted protein). Even if these drugs were subjected to protein efflux pumps, the low concentration of drugs in the cells contributes to the exertion of the required pharmacological effect. They are mechanism-based inhibitors

and thus, they are more selective because a pre-activation step is needed for the inhibition of the enzyme. Suicide inhibitors possess excellent selectivity compared with both other covalent inhibitors and non-covalent inhibitors.

On account of their mechanism-based behavior, mutations in the structure of enzymes are usually ineffective in the inactivation of these ligands. Tolerance cannot be easily developed for these inhibitors unless the corresponding enzyme becomes overexpressed. One efficient “collision” of the suicide ligand with enzyme culminates in the inactivation of the desired target, and as a result of this manner, the development of inactivating enzymes (by bacteria for example) is virtually futile. Stronger potency and extended half-life result in better cost-effectiveness and economic acknowledgment. In the case where bacteria develop a biofilm to reduce the concentration of the xenobiotics, these inhibitors would be particularly useful because only low concentration of them is required for suppressing the growth of the related microbes.

On the other hand, suicide inhibitors are harder to design because they may need a fully described enzymatic behavior for their design to provide a base-structure to target the transition-state of that enzyme. Also, the limited type of compounds deduced from those catalytic mechanisms may not be synthetically feasible for organic chemists. For a typical macromolecular target in the cells, a ligand may act as an agonist (partial or full), antagonist, or inverse agonist [57]. Suicide inhibitors result in the disruption of the active site of the enzymes, and if they have a basal activity, this would be fully suppressed by the ligand. Thus, by using suicide inhibitors, no partial inhibition can be expected. This is especially useful in the case of drug resistance when we need a quite impressive amount of diminution in the target activity. Also, ligands with suicidal behavior are only expected to prevent the normal activity of the enzyme and they cannot act as activators or agonists. Moreover, there are only a restricted number of enzymes that are capable of being targeted by a mechanism-based inhibitor compared with thousands of synthetically feasible or available compounds in databases used by medicinal chemists. There are also some immunological concerns about all covalent inhibitors and their potential immunogenicity because of their prolonged altered conformational changes they produce in their targets, which are indeed absent in most non-covalent inhibitors except those that might behave as haptens. *In silico* methods like molecular dynamics (MD) may be useful for assessment of conformational changes of protein structure, triggered by ligand molecules (both covalent and non-covalent inhibitors) and therefore assess the immunogenicity computationally [58–60]. Allopurinol, a non-selective suicide inhibitor of xanthine oxidase and other enzymes entailed in purine and pyrimidine pathways [61], displays immunogenicity and allergic reactions, putatively related to its irreversible change in the natural conformation of the enzymes. The number of discovered drugs with non-covalent inhibition massively outnumbers the number of suicide inhibitors. Table 1 summarizes our current understanding of suicide inhibitors versus non-covalent inhibitors and elaborates how these agents are superior. It is estimated that 80% of the new molecular entities (NMEs) are competing with endogenous ligands and covalent inhibitors surpass others in this sense [62].

The development of resistance (and specifically for microbial resistance) can be correlated to the probability that a mutation does not alter the activity of a certain enzyme but does reject the binding of the drug. Logically, the catalytic site of enzymes is the most intricate part of the whole structure and in this regard, they are the best “hit” for designing new drug-like molecules. Also, with the same argument, functional domains are more susceptible than structural domains, supporting motifs, loops, and related structures. Suicide inhibitors are indeed “pseudo-substrates”, and therefore for a mutation to change the binding affinity to this pseudo-substrate is, in many cases, inevitable to be concomitant with alteration in catalytic efficiency. The pressure of evolution in the widespread and injudicious usage of even a suicidal

inhibitor can eventually lead to drug resistance, as we see for one of the most common antibiotics in the world, i.e., penicillin. In the early usage of this suicidal inhibitor, penicillin was sufficiently efficacious in treating bacterial infections in world war II, and still, after 77 years of its discovery, it is widely used for treatment and prophylaxis of some infections [75].

Compared with penicillin, meropenem, a suicide inhibitor, which is prescribed only for specific diseases with resistance profile to other potent or broad-spectrum antibiotics, is considered a drug of choice for these multi-resistant pathogens including *Acinetobacter baumannii* [76], *Pseudomonas aeruginosa* [77], *Serratia marcescens* [78]. Commonly used drugs in the treatment of resistant bacterial species (the vast group of β -lactams such as penicillins, cephalosporins, monobactams, and carbapenems) exert their biological activity by using mechanism-based inhibition of their targets [72,79]. Aminoglycosides, one of the other choices for treatment of resistant infections, are not mechanism-based inhibitors, but they act as irreversible inhibitors of the ribosome complex. Fluoroquinolones, tetracyclines, and macrolides, on the other hand, are reversible binders and are not as routinely used for the treatment of multi-resistant superbugs as cephalosporins and carbapenems. Bidell et al. in 2015 reported that the resistance to the third-generation of cephalosporins is 8.6%, whereas the resistance rate for fluoroquinolones is 34.5% for urinary tract infections with *Escherichia coli* [80].

Exemestane is a mechanism-based aromatase inhibitor and anastrozole is a competitive inhibitor of that enzyme. Recent studies revealed equal potency for both of these agents and found neither of them superior to the other [73,81]. 5-fluorouracil (a suicide inhibitor [82]) of combination with carboplatin also proved to be as effective as the monoclonal antibody cetuximab in the treatment of locally-advanced head and neck cancer [83]. This example indicates that suicide inhibitors can be as effective as monoclonal antibodies (mAbs), and as a small-molecule, they are extremely more cost-effective.

Vigabatrin is a suicide inhibitor of GABA transaminase, an enzyme that degrades GABA, an inhibitory neurotransmitter, to semialdehyde and glutamate (an excitatory neurotransmitter) [84]. An Australian study represented that in 72 patients with resistant epilepsy refractory to regular anti-epileptic medications (such as phenytoin, carbamazepine, clonazepam, clobazam, barbiturates, and sodium valproate) vigabatrin displayed demonstrable efficacy in about 60% of the patients [85].

Surprisingly, selegiline, a suicide inhibitor of monoamine oxidase B (MAO-B), was established to be considerably effective for a patient in stage 5 of treatment-resistant major depressive disorder, which was treated unsuccessfully with some other medications. Imipramine, milnacipran, paroxetine, amoxapine, amitriptyline, clomipramine, lithium, and olanzapine were all impotent in assuaging the sign and symptoms of depression or resistance developed after a temporary pacification [86]. All of the ineffective medications are considered to be reversible inhibitors (although each of them has their own pharmacodynamics), but selegiline is an irreversible inhibitor with “hit and run” effect, and this may significantly contribute to the treatment success.

Not all of the suicide inhibitors are superior to reversible inhibitors. Febuxostat, a selective reversible inhibitor of xanthine oxidase, displayed superior efficacy in lowering urate levels compared with allopurinol [61]. In this case, non-selective inhibition of different enzymes involved in nucleotide metabolism conduces to lower efficiency than the selective drug febuxostat. This is again the case for the nucleoside analogue 5-fluorouracil, in which non-selective inhibition of nuclear and mitochondrial DNA dependent enzymes like DNA and RNA polymerase can engender toxic products and impairs cellular and mitochondrial function [87,88]. Hence, mechanism-based inhibition does not necessarily provide “full selectiveness” and a mechanism-based inhibitor can contribute to the (suicide or competitive) inhibition of other enzymes and drug-induced toxicity. Nonetheless, many compounds with electrophilic groups could undergo a covalent-complex

Table 1

A comparison between non-covalent inhibition and suicide inhibition.

Comparing parameters	Type of inhibition	
	Non-covalent Inhibitors	Suicide Inhibitors
Half-life of activity	Depends on the rate of drug elimination	<i>Depends on the hydrolysis of the related bond(s) or turnover rate of the enzyme (therefore significantly higher)</i>
Efflux potentiality of the cells	Depends on being the substrate of the corresponding enzymes	<i>Independent, since they only need to “meet” their targeting enzyme once</i>
Susceptibility to inactivation by changes in the structure of receptors	Simply inactivated by mutations in the receptor	<i>More resistant to mutations in the enzyme because they are mechanism-based</i>
Susceptibility to inactivation by development of inactivating enzymes	Could be the substrate of inactivating enzymes	<i>More resistant since one confrontation results in the inactivation of the receptor</i>
Selectivity	Based on the structure of the ligand a wide spectrum of selectivity can be achieved	<i>Highly selective owing to their mechanism-based nature and pre-activation by the enzyme [63]</i>
Economic & commercial suitability	Based on the synthetic feasibility and the potency of selected drug varies	<i>Higher rate of inhibition requires lower amount of drug and lower cost [64]</i>
Tolerance & Tachyphylaxis	Easily developed via arrestin, overexpression, mutations, etc.	<i>Practically not applicable (unless the enzyme becomes overexpressed)</i>
Level of efficacy	Can be full agonists, partial agonists, antagonists or inverse agonists	<i>Fully inactivates the related enzyme; there is no control over the level of their inhibition (either they are antagonists or inverse agonists)</i>
Synthetic feasibility	<i>Can be more readily synthesized or analogues can be developed more easily</i>	A narrow-spectrum of compounds could act as suicide-inhibitors and harder to synthesize
Difficulty of design	<i>Can be accomplished using in silico rational drug design</i>	Harder, because they should be based on the catalytic activity of the enzyme [59]
Target finding for drug design	<i>Easy, there are hundreds or even thousands of protein that can be targeted for a specific disease</i>	Troublesome, a mechanism-based inhibitor usually needs an enzyme with a defined catalytic activity to be targeted [65]
Immunogenicity	<i>Usually non-immunogenic unless they act as haptens</i>	There are concerns about their immunogenicity because they can alter the normal structure of the enzyme [66]
Toxicity	<i>Generally more tolerable and safer</i>	Typically more toxic, the toxicity should be adjusted by lower doses [67]
Number of drugs with this type of mechanism	<i>Several thousands</i>	Less than one hundred [63,68]
Examples of drugs currently used	Erythromycin [69], anastrozole [70], moclobemide [71]	Penicillin [72], exemestane [73], selegiline [74]

with that ligand and act as suicide substrates, but fitting into the enzyme active site is the crucial step in the design of new efficient inhibitor of mechanism-based inhibitors.

β -lactams, vigabatrin, exemestane, and selegiline cases comparison suggests that suicide inhibitors are also useful for the cases of drug resistance that rapid cell division (like cancer cells, bacterial cells, and viruses) is not involved.

Consequences of the hypothesis

Drug resistance is ubiquitous and providing better therapeutic with lower resistance not only helps to combat infectious diseases, but also many other pathological diseases which there is a lack of efficient therapeutic. Cancer, of the main human diseases, is perhaps the most salient pathological condition that stands to benefit from drugs with lower or nearly zero drug resistance. Even diseases with genetic etiologies would be quenched with these effective drugs. Lower cost (due to very lower doses required for treatment) may be the advantage of suicide inhibitors with respect to other traditional medicines and this could be helpful for both pharmaceutical industries and patients. Finally, from a well-being perspective, this could save the lives of thousands or millions of people and prolong their longevity.

Conclusion

Irreversible inhibitors usually contain an electrophilic functional group like α,β -unsaturated ketones, vinyl sulfones, α -substituted carbonyl derivatives, bromodihydroisoxazoles, etc., which react with the nucleophilic group of the active site [89]. Mechanism-based inhibitors, as their name implies, are designed to be pre-activated by the enzyme and then produce an inoperative enzyme-drug adduct. Suicide inhibitors usually have a leaving group, replaced by a nucleophile in the enzyme active site that forms a stable transition state complex with the enzyme. The concerns about the possible toxicity of these agents have

pushed them as a second or third choice for medicinal chemists, but in the case of drug resistance, they can provide many advantages over conventional and frequently used non-covalent inhibitors. For enzymes that possess a basal activity (like adenylate cyclase), suicide inhibitors may act as inverse agonists, which thoroughly switch off the enzyme and provide greater efficacy than a strong antagonist. This is one of the other important advantages of these inhibitors compared with other non-covalent inhibitors, which is not fully described in the literature so far. Finally, it is worth mentioning that a suicide inhibitor could act as suicide or competitive inhibitor of the structurally similar enzymes, and in this way, selective fitting of the ligand into the active site of the enzyme is fundamentally vital for high-selectivity and a lower rate of resistance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2020.109676>.

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