Review

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Resistance-resistant antibiotics

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New antibiotics are needed because drug resistance is increasing while the introduction of new antibiotics is decreasing. We discuss here six possible approaches to develop 'resistance-resistant' antibiotics. First, multitarget inhibitors in which a single compound inhibits more than one target may be easier to develop than conventional combination therapies with two new drugs. Second, inhibiting multiple targets in the same metabolic pathway is expected to be an effective strategy owing to synergy. Third, discovering multiple-target inhibitors should be possible by using sequential virtual screening. Fourth, repurposing existing drugs can lead to combinations of multitarget therapeutics. Fifth, targets need not be proteins. Sixth, inhibiting virulence factor formation and boosting innate immunity may also lead to decreased susceptibility to resistance. Although it is not possible to eliminate resistance, the approaches reviewed here offer several possibilities for reducing the effects of mutations and, in some cases, suggest that sensitivity to existing antibiotics may be restored in otherwise drug-resistant organisms.

The rise of antibiotic resistance

With the recent issuance by the US Centers for Disease Control and Prevention (CDC) of its *Threat Report 2013* on antibiotic resistance [1] and the World Health Organization (WHO) *Antimicrobial Resistance: Global Report on Surveillance 2014* [2], there can be little doubt that antibiotic resistance will be a major public health threat for the foreseeable future. This threat is recognized, for example, by the recent announcement of the (~$17 million) Longitude Prize 2014 (http://www.longitudeprize.org/) on the 300th anniversary of the Longitude Act 1714 (the Prize was won half a century later by John Harrison, for his chronometer), the current topic being: 'How can we prevent the rise in resistance to antibiotics?' More recently, President Obama signed an Executive Order: Combating Antibiotic-Resistant Bacteria (http://www.whitehouse.gov/the-press-office/2014/09/18/executive-order-combating-antibiotic-resistant-bacteria), offering a $20 million prize in the same general area as the Longitude Prize, focus in both cases being in the area of diagnostics. We review here some of the scientific possibilities for resistance-resistant antibiotics.

Antibiotics have been known for hundreds and, in some cases, thousands of years – albeit not in pure form. For example, the 'newest' antimalarial in widespread use, artemisinin (1 in Figure 1), was used in traditional Chinese medicine ~2000 years ago [3] but was only isolated in a pure form in the 1970s by Tu Youyou [4–6] apparently owing to a request from Ho Chi Minh to Mao Zedong for an antimalarial to replace chloroquine (**2**) (developed in WWII), where resistance had developed. The antimalarial quinine (**3**), whose activity (in cinchona bark) was known in the 15th century by the Incas, was later brought to Europe and quinine was isolated, by French scientists, in 1820 [7]. However, the mechanisms of action of all three drugs have been the topic of debate, suggesting, perhaps, that they might each have more than one target.

The first development of a synthetic antibiotic began about 100 years ago with the discovery of Salvarsan (compound 606; Arsphenamine) by Ehrlich [8], proposed in early work to be **4**. However, the actual structures of Salvarsan **5**, **6** were only discovered a decade ago [9] and the exact targets, presumably one or more proteins containing reactive thiols to which arsenic can bind, are still unknown. The next generation of antibiotics, sulfonamides such as Prontosil (**7**), were developed by IG Farben [10] and their target, dihydropteroate synthase, is known, but resistance occurred rapidly and the use of sulfonamides was largely replaced by the discovery and development of penicillin **8** (which does have multiple targets), shortly before and during WWII.

After the development of penicillin there was a 'golden age' of antibiotic discovery during the 1950s and 1960s, however, after ~1985 there has been a sharp fall-off in new antibiotic drug discovery for reasons that are at least in part financial; an antibiotic that cures a patient in perhaps one week does not have a large profit potential. However, perhaps more importantly, it does appear that many of the developments in high-throughput screening (HTS) of synthetic libraries, genomics, structure-based design, combichem, and natural product screening have led to relatively few new progressable leads. While it is certain that chemists need to improve their chemical libraries, this is in a sense a chicken/egg problem in that, if it was clear how to do this, we would likely already have more antibiotics! Indeed, as discussed in two recent reviews [11,12], most of the recently introduced antibiotics belong to existing antibiotic classes. Unfortunately, during this same time-period drug resistance with for example MRSA (methicillin-resistant *Staphylococcus aureus*) or multidrug-resistant as well as extensively drug-resistant *Mycobacterium tuberculosis*, multiple cases of hospital-acquired infections (*Clostridium difficile*, and infections with Gram-negatives such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*) have occurred and are

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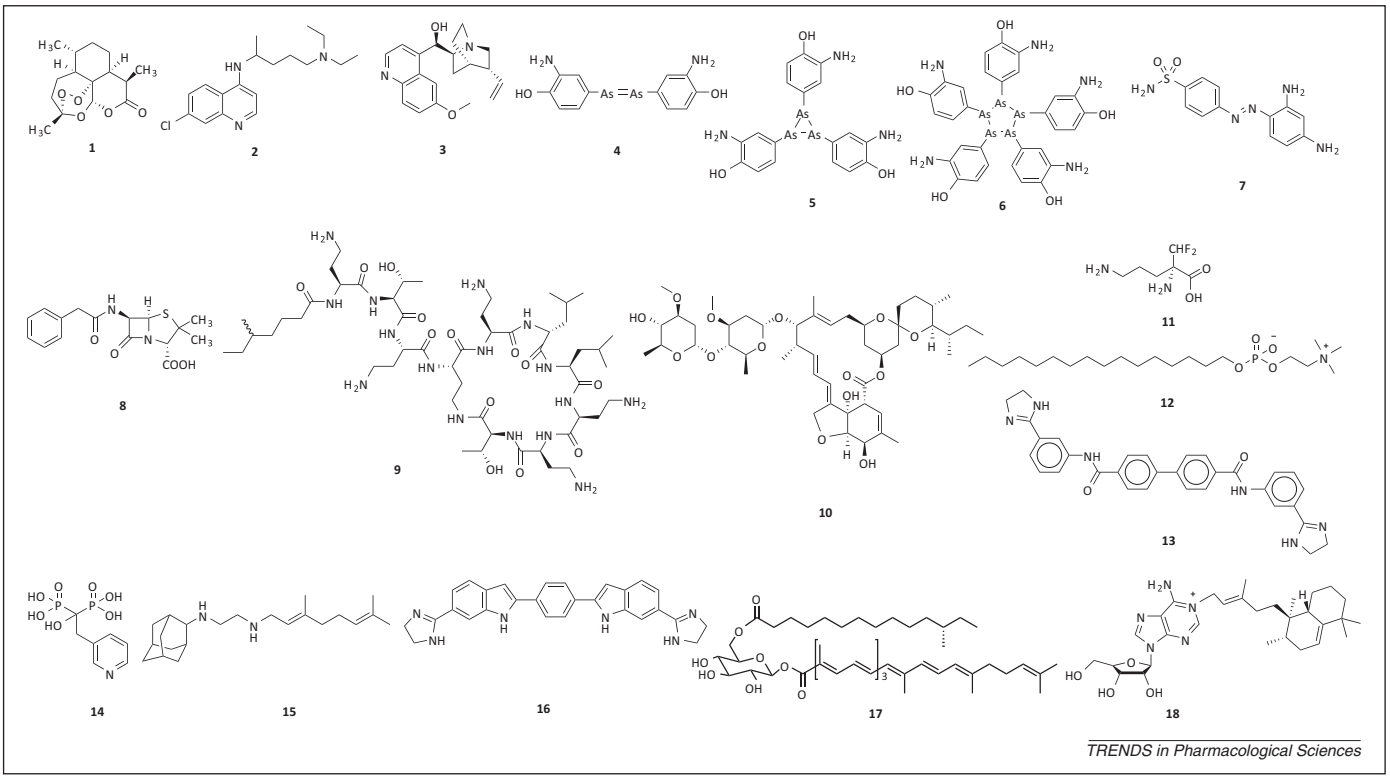

Figure 1. Structures of some drugs, drugs leads and other compounds of interest. **1**, artemisinin; **2**, chloroquine; **3**, quinine; **4**, early proposed structure of Salvarsan; **5**, **6**, actual structures of Salvarsan; **7**, Prontosil; **8**, penicillin; **9**, colistin; **10**, ivermectin; **11**, eflornithine; **12**, miltefosine; **13**, bisamidine, BPH-1358; **14**, risedronate; **15**, SQ109; **16**, MBX-1066; **17**, staphyloxanthin; **18**, tuberculosinyl adenosine.

anticorrelated with the introduction of new anti-infectives [13,14], although apparently there is now renewed interest in the development of new antibiotics by the pharmaceutical industry [15].

This lack of new antibiotics has been highlighted in the CDC and WHO reports, although such warnings are not new [16] and indeed were basically presaged by Fleming [17]. What is new is the discovery of organisms such as *Klebsiella pneumoniae* producing both NDM-1 carbapenemase and β-lactamase, leading to the strong possibility of pan-resistance and in some cases, the reintroduction of an old drug, colistin (**9**; a polymyxin), a polycationic species that targets bacterial cell membranes, for treating such infections. In addition, there is now a report of the occurrence of vancomycin resistance in MRSA *S. aureus* [18]. Why these developments are so alarming is that patients undergoing any surgical procedure – for cancer, heart disease, joint replacements, for example – will be at greatly increased risk for acquiring an untreatable bacterial infection, not least because in many instances they will already have a compromised immune system.

In the less-developed world, HIV/AIDS, malaria, tuberculosis, as well as the leishmaniases and Chagas disease, cause millions of deaths and hundreds of millions of DALYs (disability adjusted life years) [19], mostly in Africa, and once again there have been few new drugs introduced because the profit motive is even less attractive for patients who have few (or no) resources. Some success stories have, however, been reported. For example, the drug ivermectin **10**, developed to treat heartworm in dogs, is used to treat river blindness; the facial hair removal drug Vaniqua (eflornithine, **11**) initially developed as an anticancer drug, has been used to treat sleeping sickness [20] and another anticancer drug lead, miltefosine **12**, is now FDA-approved to treat the leishmaniases (http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm389671.htm), the common theme here being that none of these drugs/drug leads were originally developed to treat the neglected tropical diseases but were later found to have activity against parasitic protozoa, and in some cases have been provided by pharmaceutical companies at low or no cost to patients. These developments clearly show an important role for drug repurposing, a topic we discuss below.

In the following we therefore enumerate and expand on a series of six general propositions, with examples, that may help to facilitate the development of new therapeutic strategies and leads. The focus is largely though not exclusively on new applications of multitarget inhibitors in infectious diseases (bacterial and protozoan), based in part on new concepts and discoveries as to how some drugs/drug leads function.

Multitarget inhibitors

For drugs with a single mechanism of action, combination therapies are needed to combat resistance. However, there are intrinsic difficulties associated with developing multiple new drugs for combination therapies: multitargeting might be easier. In many cases, the root cause of antibiotic resistance is that a mutation occurs in the target, usually a protein, rendering an inhibitor ineffective. In some cases larger inhibitors can circumvent resistance due to enhanced drug–target interactions (as with e.g., the