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# Antimicrobial Resistance and the Repurposing of Primaquine: A Review of Design, Screening and Antibacterial Evaluation

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**Abstract**

Antimicrobial resistance (AMR) has become one of the most serious threats to modern healthcare. Bacteria that were once easily treated now survive the antibiotics designed to kill them, and the pipeline of genuinely new antibacterial drugs has slowed almost to a halt. One practical way forward is to take a drug that is already well understood in humans and modify its structure so that it acts on bacteria instead. Primaquine, an 8-aminoquinoline licensed as an antimalarial since 1952, suits this approach because it carries a simple terminal amine group that chemists can readily convert into new ring systems. This short review explains why AMR has become so difficult to contain, describes the main ways bacteria defend themselves against antibiotics, and summarises what has been learned from two recent studies on primaquine derivatives. In the first, one hundred derivatives were designed and screened by computer against targets from multidrug-resistant tuberculosis, methicillin-resistant Staphylococcus aureus and resistant Escherichia coli, and ten were selected as safe and promising. In the second, five of those ten were prepared in the laboratory, confirmed by spectral analysis and tested against both normal and resistant bacteria. The derivatives inhibited the resistant strains at concentrations several times lower than primaquine itself, while the standard reference antibiotics gave no measurable endpoint at all. Together the studies show that a simple chemical modification can turn a stage-selective antimalarial into a broad antibacterial lead.

**Keywords:** Antimicrobial resistance, Primaquine, Drug repurposing, MRSA, MDR-TB, Molecular docking, Thiazolidinone.

## Introduction

For most of the twentieth century, infectious disease appeared to be a problem that medicine was steadily solving. In 1900 roughly one third of all deaths were caused by infection. By 2014 the leading causes of death in most countries were non-communicable conditions such as heart disease and cancer. Better sanitation, better nutrition and, above all, the arrival of penicillin and the antibiotics that followed it were responsible for this change, and for a time it was reasonable to believe that bacterial infection would eventually be eliminated altogether [1, 2].

That expectation has not been met. Infectious diseases are increasing again, and the infections that are increasing fastest are the ones caused by bacteria that no longer respond to the antibiotics developed to treat them. Methicillin-resistant Staphylococcus aureus (MRSA) is associated with close to 50,000 deaths each year in the United States and Europe alone. More than 480,000 cases of multidrug-resistant

tuberculosis were recorded in a single year, a burden carried mainly by developing countries [3, 4]. If no effective action is taken, antimicrobial-resistant infections are projected to cause about ten million deaths every year by 2050, with an associated economic loss of the order of 100 trillion US dollars [5, 6].

The scale of the problem has been recognised internationally. The World Health Organization adopted a Global Action Plan on antimicrobial resistance in 2015, and individual countries were asked to prepare their own national action plans. India released a five-year National Action Plan on AMR in April 2017, setting out its containment targets and the strategies for meeting them [7, 8]. India is important in this context because it currently reports among the highest rates of antibiotic resistance in the world. It is also worth noting that AMR is not only a hospital problem. The One Health principle recognises that human health, animal health, food production and the environment are connected, and that antibiotics used in agriculture and released into the environment contribute to resistance just as clinical use does [9].

**Mechanisms of AMR**

To understand why new chemical scaffolds are needed, it helps to understand what bacteria actually do to survive antibiotic treatment. Four mechanisms account for most clinical resistance: enzymatic destruction of the drug, active removal of the drug from the cell, modification of the target the drug binds to, and reduced permeability of the cell envelope. The first two are the most widespread and are described below; the latter two operate by preventing the drug from ever reaching or recognising its target [13].

The first and most common is enzymatic destruction. Bacteria produce enzymes that chemically break the antibiotic before it can act. The beta-lactamases are the best-known example. These enzymes cleave the four-membered beta-lactam ring at the heart of the penicillins, cephalosporins, monobactams and carbapenems, and they are divided at the molecular level into four classes, A, B, C and D. Class B enzymes require a zinc ion and are therefore metalloenzymes, while classes A, C and D operate by a different chemistry. Class D enzymes, produced by Gram-positive cocci including Staphylococcus aureus, are able to degrade oxacillin [10, 11].

The second mechanism is active removal. Bacteria possess energy-dependent pumps that expel the drug from the cell as fast as it enters, so that a lethal concentration is never reached inside. This is the principal resistance route for the tetracyclines, and similar pumping systems act against quinolones, chloramphenicol and beta-lactams. The genes involved may sit on the chromosome or on plasmids, which allows the trait to spread between organisms [12].

The practical lesson is that resistance is usually directed at a particular chemical family rather than at antibacterial action in general. A beta-lactamase recognises the shape of the penicillin nucleus. An efflux pump recognises a particular class of molecule. This is why introducing a genuinely different chemical scaffold, rather than making yet another variation on an existing one, is one of the more promising responses to resistance [15, 16].

**Primaquine as a Candidate Scaffold for Structural Modification**

Primaquine is an 8-aminoquinoline that has been licensed by the United States Food and Drug Administration since 1952. Sixty years after its approval it still holds an uncontested place in antimalarial therapy, because it remains the only licensed medicine proven effective against the dormant liver stages, the hypnozoites, of Plasmodium vivax and Plasmodium ovale [14]. Its mechanism is not fully understood, but it is thought to interfere with parasite respiration by generating reactive oxygen species and slowing electron transfer.

From a chemist's point of view, however, the important feature of primaquine is structural. The molecule carries a 6-methoxyquinoline nucleus joined at its C-8 position to a flexible pentyl chain that ends in a free primary amine. A free primary amine is one of the most convenient groups in synthetic chemistry: it reacts readily with aldehydes to form an imine, or Schiff base, and imines are standard starting points for building new ring systems. In effect, primaquine offers a stable, well-characterised drug core with a ready-made chemical handle attached to it. Modifying that handle allows the biological behaviour of the molecule to be redirected while leaving the quinoline core intact. Before the studies described below, no primaquine derivatives had been reported against antibacterial-resistant organisms at all.

**In Silico Design, Molecular Docking and ADMET-Based Screening**

A previous study took a computational approach [17]. One hundred new primaquine derivatives were designed on paper, chosen so that each could realistically be made in the laboratory from the Schiff base intermediate. These structures were then docked, using the CDOCKER method in Discovery Studio, into three bacterial protein targets chosen to represent the three resistance problems of greatest concern in India: multidrug-resistant tuberculosis, MRSA and antibiotic-resistant Escherichia coli. Primaquine itself, along with amoxicillin, cefixime and ciprofloxacin, was docked under identical conditions so that the derivatives could be judged against a fair benchmark.

Docking predicts how well a molecule fits into the binding pocket of a protein and how strongly it is likely to be held there. Fifty of the hundred derivatives bound more strongly than the reference drugs and were carried forward. These fifty were then assessed computationally for their likely behaviour in the body, using absorption, distribution, metabolism, elimination and toxicity descriptors together with TOPKAT toxicity models. This second filter asked practical questions: would the compound be absorbed from the gut, would it dissolve adequately, would it damage the liver, would it interfere with drug-metabolising enzymes, and would it show carcinogenic or mutagenic behaviour?

Ten compounds survived both filters, showing good predicted absorption and solubility, no predicted liver toxicity and acceptable toxicity profiles. Their binding poses were also informative: the strongest binders did not owe their affinity to more hydrogen bonds. Primaquine itself forms clean hydrogen bonds in each site but makes little other contact, whereas the best derivatives made a very large number of weaker contacts at once, using their extended aromatic and alkyl groups to fill the binding pocket. The improvement therefore came from better overall shape complementarity rather than from any single strong interaction.

**Synthesis, Structural Characterization and Antibacterial Evaluation**

Computational prediction is only useful if the molecules can be made and tested, and the second study did exactly that [18]. Five of the ten prioritised compounds were prepared in the laboratory. Primaquine was first condensed with the appropriate aldehyde in acidified methanol to give the Schiff base. That intermediate was then treated with thioglycolic acid in dioxane, in the presence of a small quantity of anhydrous zinc chloride, to close a five-membered 4-thiazolidinone ring. The products were recrystallised from ethanol and their structures confirmed by infrared spectroscopy, proton and carbon-13 NMR and mass spectrometry, with melting points recorded for each.

The compounds were then tested against six bacterial strains: wild-type Escherichia coli, Staphylococcus aureus and Mycobacterium tuberculosis, together with their resistant counterparts, namely ciprofloxacin-resistant E. coli, MRSA and multidrug-resistant tuberculosis. Minimum inhibitory concentrations were

determined by the standard broth dilution method, and primaquine, ciprofloxacin, methicillin and amikacin were included for comparison.

The results were encouraging in a specific and important way. All five derivatives inhibited the susceptible strains at concentrations in the region of 70 to 125 micrograms per millilitre, and the resistant strains at concentrations in the region of 120 to 160 micrograms per millilitre. Primaquine itself required considerably higher concentrations, in the range of 300 to 550 micrograms per millilitre against the resistant organisms. The reference antibiotics gave no measurable endpoint against the resistant panel at the concentrations examined, which is exactly what one expects of ciprofloxacin against a ciprofloxacin-resistant isolate and of methicillin against MRSA. This absence of a comparator endpoint served as an internal check that the resistant phenotypes were genuine.

The most instructive observation is the gap between the two sets of numbers. Against ordinary E. coli, primaquine is only slightly weaker than its derivatives. Against the resistant strains the difference widens to roughly four-fold. This pattern suggests that the chemical modification does not simply make the molecule generally more potent; it makes the molecule less vulnerable to whatever resistance mechanism the bacterium is using. That is precisely the property a new antibacterial scaffold needs to have.

**Discussion and Future Perspectives**

The two studies form a complete and internally consistent sequence: design, computational screening, safety filtering, synthesis, structural confirmation and biological testing. Every compound that was predicted to work and then made did in fact show activity, which is a reasonable validation of the screening approach even if the precise ranking produced by docking should not be over-interpreted.

Three lines of work follow naturally. First, the remaining prioritised compounds should be synthesised and tested so that the full set is experimentally characterised rather than only half of it. Second, in vivo pharmacokinetic, pharmacodynamic and toxicity studies are needed to establish whether the favourable computational predictions hold in a living system. Third, the actual mechanism of antibacterial action needs to be determined, since the docking studies propose a binding target but do not prove that the observed killing occurs through it.

**Conclusion**

Antimicrobial resistance is advancing faster than conventional antibiotic discovery can respond, and chemically distinct scaffolds are needed rather than further variations on existing families. Primaquine offers an attractive starting point because it is a long-established medicine with a convenient reactive site that allows its biological behaviour to be redirected. Two linked studies have shown that this idea works in practice: one hundred derivatives were designed and narrowed by computational screening and safety prediction to ten promising candidates, and five of these were then synthesised, structurally confirmed and shown to inhibit MRSA, multidrug-resistant tuberculosis and ciprofloxacin-resistant E. coli at concentrations well below those required by primaquine, under conditions in which the standard reference antibiotics were inactive. These are early-stage results, and the remaining compounds, the mechanism of action and the behaviour of the series in vivo all require investigation. Even so, the work demonstrates that a familiar antimalarial can be redirected against resistant bacteria by a straightforward chemical modification, and that repurposing of this kind deserves a place alongside conventional discovery in the response to antimicrobial resistance.

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