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3.4 Synthetic Vs. Natural Bioactive Compounds Against Tropical Disease

3.4.1 Introduction

Tropical diseases, although largely neglected by the commercial pharmaceutical industry, are a major problem for people in poor and underdeveloped nations. Perhaps the most significant of these threats comes from malaria. Roughly half of the world's population live in malaria endemic regions, and the WHO estimates that it caused 627,000 deaths in 2012 (CDC, August 18, 2014). Nearly 500 million cases of malaria are confirmed worldwide annually, with roughly 80% of those caused by *Plasmodium vivax*, a persistent strain of the parasite (Goncalves, 2014). Due to the constant emergence of resistance, there is an ongoing need for the development of new drugs for malaria (Rosenthal, 2003). For this reason, and in the interest of outlining a generic drug development paradigm in limited space, we will focus the topic of the current discussion on the historical successes and failures of natural products in treating malaria and the efforts in the modern era to combat drug resistance and poor drug tolerance with synthetic drugs.

3.4.2 Early History of Malaria Treatment; Quinine and Artemisinin

"The angel of disease and death, ascending from his oozy bed, along the marshy margins of the bottom grounds [...] floats in his aerial chariot, and in seasons favorable to his progress, spreads mortal desolation as he flies", so it was written in an Ohio newspaper article from 1820 (Findley, 1968). From this quote one can see how parasitic disease plagued the early frontiersmen of the 19th century. However, the unhappy coexistence of humans and parasitic disease goes back much further into the past. In fact, the earliest recorded descriptions of a disease with symptoms consistent with those of malaria date back to 2700 BC from imperial China. During this period traditional Chinese medical practitioners discovered the use of sweet wormwood (*Artemesia annua*) to treat this mysterious fever. Meanwhile, on the other side of the planet Quechuas from South America used the bark of the cinchona tree in the treatment of similar fevers. Jesuit monks brought this bark from the new world and introduced

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its powerful medicinal properties to Europe during the 17th century, however, the active component, quinine, was not isolated until 1820 (Faurant, 2011). Quinine is known for several unpleasant and potentially severe adverse reactions including cinchonism, hearing impairment, increased risk of hemolysis in G6PD deficient patients, and is linked to a severe syndrome in some patients, dubbed blackwater fever. Modern efforts suggest the latter may be the result of redox active metabolites (Marcsisin, 2013). As we will explore, many of the ongoing efforts in anti-malarial therapy revolve around not only circumventing resistance, but in mitigating many of the safety risks associated with historically used drugs. These reasons and the outbreak of war in malaria endemic regions of the world led to a surge of interest in the development of safe and effective drugs to treat malaria that we will explore further.

3.4.3 Post World War II and the Development of Synthetic Anti-malarials

"Doctor [...] this shall be a long war if for every division I have facing the enemy I must count on a second division in the hospital with malaria and a third division convalescing from this debilitating disease!" Thus opined Gen. Douglas MacArthur concerning the ravages of malaria during the second world war (Coates, 1963). This constant struggle with non-combat related injury from disease was a tremendous burden on medical logistical chains, morale, and overall readiness on fronts across Africa, Asia, and even in parts of Europe. This reality was a major shaping force for a massive effort to prevent and treat malaria.

During WWI, Germany had no access to quinine. As a result, a large-scale effort ensued in the intervening years between WWI and WWII in which the Germans synthesized and screened thousands of compounds for anti-malarial activity. Among these were several 8-aminoquinolines, atabrine (quinacrine), and a drug they discarded for toxicity called Resochin (chloroquine) (Coates, 1963; Vale, 2009). After access to quinine from Indonesia was cut to Allied forces by the entry of Japan into WWII, US and British chemists began a similar push resulting in the adoption of atabrine as the drug of choice for malaria treatment and prophylaxis during the later years of the war. Post-war, powerful new anti-malarial drugs began to emerge as the culmination of follow-on efforts by US and British chemists to exploit the original efforts by Germany. Many of these drugs are still in use today, with robust analog efforts being employed around them to overcome developing resistance.

3.4.4 Modern Efforts in Antimalarial Drug Development

Modern efforts in anti-malarial drug discovery in general follow six separate approaches including optimization of therapy with existing agents, development

of analogs of existing agents, natural products, repurposing of drugs from other therapeutic areas, reversal of resistance, and discovery of compounds active against novel targets (Rosenthal, 2003). Table 3.4.1 illustrates examples from current literature from each approach. It is interesting to note that many of these approaches revolve around existing agents and their analogs, many of which were known since the 1920s, 30s, or 40s. For the purposes of this discussion, we will focus on ongoing work in existing classes and development of novel classes of anti-malarials, as discussions of combinations of existing drugs can become lengthy and are better suited in a clinical pharmacology text.

3.4.4.1 Quinine, 4-Aminoquinolines, and Quinoline Methanols

For natural products, quinine is a success story that has endured for centuries. Quinine is an aryl amino alchohol derived from the bark of the cinchona tree (Fig. 3.4.1). Despite its discovery over 400 years ago, it remains an enormously important drug in the treatment of malaria in the developing world. Quinine is rapidly absorbed, both orally and parenterally, broadly distributed throughout the body, and has proven highly effective for the treatment of uncomplicated or severe malaria (Achan, 2011). In the age of resistance, quinine has made a resurgence in usage. Although quinine resistance has been reported, it is generally low-grade, and is largely found in Asia and South America (Noedl, 2006; Parola, 2001).

Perhaps a larger issue still in play for quinine is tolerance. Quinine has several notable and potential severe adverse events associated with usage. Most common side-effects include tinnitus and hearing impairment, but more severe effects can include vertigo, vomiting, abdominal pain, or hypotension (Achan, 2011). The most severe and potentially least understood is a syndrome called blackwater fever characterized by hemolysis and hemoglobinuria (George, 2009). Recent studies suggest a potential link between CYP mediated oxidative metabolites of quinine and this potentially fatal reaction (Marcsisin, 2013). It should also be noted that while clinically different from the hemolytic events observed with the 8-aminoquinolines (which we will discuss in a later section), as with the 8-aminoquinolines a link may exist between glucose-6-phosphate (G6PD) deficiency and hemolysis (Hue, 2009). This is significant as it suggests common metabolic pathways for some quinoline drugs that should be avoided or at least considered when developing new drugs in these classes. For quinine, the formation of redox active quinones may lead to increased oxidative stress under conditions of high parasitemia or in G6PD deficient individuals that ultimately results in the destruction of red-cells through mechanisms which are not fully understood (Fig. 3.4.2) (Marcsisin, 2013).

Arguably one of the most successful classes of drugs to arise from WWII efforts is the 4-aminoquinolines. Fig. 3.4.3 illustrates two members of the class, chloroquine and amodiaquine, and can serve as a generic paradigm for its structure. Chloroquine was

originally discovered by Hans Andersag in 1934 while working for Bayer, however, the drug was discarded for perceived toxicity. After its re-discovery by British and American chemists, it quickly won favor as a safe and effective anti-malarial (CDC, August 18, 2014). In fact, chloroquine was used extensively in post-war eradication efforts. While the mechanism of action for all 4-aminoquinolines is not exactly known, they are known to be effective in treating only erythrocytic stages of infection. Poor compliance or poor management of care with such drugs can rapidly lead to resistance. This was exactly the case with chloroquine. By the early 1950's, chloroquine resistance was identified along the Thai-Cambodian border and Colombia. Within two decades it had spread to every malaria endemic region of the world (Farooq & Mahajan, 2004).

While the mechanism of action for the 4-aminoquinolines is not completely understood, the most widely cited hypothesis is that accumulation in the digestive vacuole of the parasite interferes with hemoglobin digestion. Resistance is believed to be conferred by the presence of a chloroquine specific P-glycoprotein pump (Foley & Tilley, 1998). Several loci in the *P. falciparum* genome have been implicated in this resistance (Farooq & Mahajan, 2004). This mechanism, or a variant thereof may well be important in quinine, mefloquine, or other quinoline resistance mechanisms, however, it should be noted that more lipophilic quinolines do not concentrate in the food vacuole to the same extent as chloroquine, and therefore other mechanisms need to be explored (including potential alternative targets of efficacy) when considering these drugs.

A considerable effort is still ongoing to circumvent resistance through analog campaigns or co-administration with other drugs (Hanboonkunupakarn, 2014; Le Garlantezec, 2014; I. Opsenica, 2011; I. M. Opsenica, 2013; Thriemer, 2014). While it is tempting to synthesize analogs of a compound with emerging or established resistance, in the author's opinion, such efforts should be entered into with full knowledge that selection pressures in malaria endemic areas quickly erode the efficacy of new drugs from old classes. It is advisable to pursue a combination approach wherein fast acting short half-life drugs (e.g. artemesinins) are combined with long half-life (longer exposure of parasite to drug) quinolines prone to emergence of resistance. Further, future efforts in this class should pay careful attention to early signs of cross resistance in chloroquine resistant strains. While small jumps in IC50 in a resistant strain may not raise alarms if those IC50s are still significantly less than the anticipated maximum concentrations achieved in plasma post-exposure, they could be indicative of shared mechanisms of resistance that might worsen over time with large exposures in environments with poorly controlled administration or where monotherapy is used.

Mefloquine, a quinoline methanol, was developed by the US Army in the 1970s, and was considered very desirable for both treatment and prophylaxis due to its long half-life (Croft, 2007). In recent years mefloquine usage has decreased due to emerging resistance, and more significantly, poor tolerance (Farooq & Mahajan, 2004; Milner, 2010). Mefloquine has been linked to neurological side-effects including

vertigo, loss of balance, and polyneuropathy, which have recently been labeled as potentially irreversible by the FDA (Nevin, 2014). Extensive efforts have shown promise in improving the therapeutic index of mefloquine by opening or removing the piperadine side-chain (Dow, 2006; 2011; Milner, 2011). With the notoriously poor predictive power of models of CNS toxicity and the stigma attached to the quinoline methanol class, these efforts have largely been abandoned. Further, recent trials with enantiomerically pure mefloquine (currently marketed as a racemic mixture) have shown little promise in improving tolerability (Nevin, 2014).

3.4.4.2 8-Aminoquinolines

Another powerful class of anti-malarial drugs to emerge from war efforts is the 8-aminoquinolines. Although still quinoline based, this class has several key features which make it unique. One primary feature of this class is its ability to act as a causal prophylactic, or to prevent the initial infection of parasites in the liver. Further, the class has powerful anti-hypnozoite activity. Hypnozoites being the dormant liver stage of *P. vivax* and *P. ovale*, this imparts a separate prophylactic use, namely presumptive anti-relapse therapy or PART. Combined with this unique exoerythrocytic activity is the gametocytocidal activity, whereby 8-aminoquinolines kill the sexual stages of the parasite blocking further transmission of infection. This combination of activities makes this class highly attractive for prophylaxis, treatment of relapsing strains of malaria, and/or elimination efforts. The class has also shown *in vitro* anti-leishmanicidal activity and has clinical utility in the treatment of pneumocystic pneumonia, and trypanosomiasis (Vale, 2009). However, as with other classes that we have reviewed here tolerability is a substantial problem for the 8-aminoquinolines. From this class, currently only primaquine is clinically available for these uses.

Administration of 8-aminoquinolines is known to cause methemoglobinemia and hemolysis in G6PD deficient individuals. Both of these effects are linked to oxidative metabolites rather than the parent drug itself (Ganesan, 2009; Ganesan, 2012). Interestingly, these same metabolites were recently shown to play a crucial role in activity (Bennett, 2013; Marcsisin, 2014; Pybus, 2013). For both primaquine and tafenoquine (8-aminoquinoline, currently in clinical development), activity is mediated by CYP 2D6 (Fig. 3.4.2). The mechanism of activity for the 8-aminoquinolines is not fully understood, however, contemporary thought is that redox active metabolites produce peroxides and superoxides (oxidative stress), which ultimately interferes with electron transport in the parasite. Not surprisingly, what is also unknown at present is whether the toxicity can be mitigated while preserving efficacy. Work in this area is of paramount importance, as new 8-aminoquinolines with similar profiles to primaquine are of little clinical utility.

The WHO currently calls for a 30 mg dose of primaquine as a chemoprophylaxis in areas where *P. vivax* is the predominant risk, however significant fractions of the

population (10% in Europeans and as much as 50% in some Asians) possess 2D6 alleles with reduced function (Deye & Magill, 2014). It is therefore likely that primaquine prophylaxis and even anti-relapse therapy will fail in many of these individuals. All things considered, the importance of this class cannot be overemphasized and it cannot be abandoned without more study. As stated previously, primaquine is currently the only clinically available drug for the treatment of relapsing malaria. As such, the challenges posed for future 8-aminoquinoline development include re-routing metabolism through a non-CYP 2D6 pathway, circumventing hemolytic toxicity, or both. The primaquine enamine bulaquine showed some promise in recent animal studies at improving the therapeutic index (Lal, 2003; Mehrotra, 2007). This appears to be due to differential pharmacokinetic exposure patterns as compared to parent primaquine. The addition of the enamine group on the side-chain creates an almost time-release effect as it is hydrolyzed to release primaquine. This leads to an interesting hypothesis that perhaps while the same metabolites may well be responsible for efficacy and toxicity, efficacy could be exposure driven while toxicity is concentration dependent, providing an in-road to improvements in therapeutic index. Although in the author's opinion, circumstantial evidence overwhelmingly points to an inextricable link between efficacy and toxicity in this class that may make it difficult, if not impossible, to support the large-scale development of a new candidate.

3.4.4.3 Artemisinins and other Endoperoxides

Although sweet wormwood was used for over 2000 years in China to treat malaria, the active ingredient, artemisinin, was not identified until the 1970's (Fig. 3.4.5) (Faurant, 2011). At present, several artemisinin compounds are clinically available for use including artemisinin, artesunate, dihydroartemisinin (DHA), and artemether. Other experimental drugs exist but are not clinically available. Due to poor tolerability and hence compliance with quinine, artemisinin compounds have drastically risen in popularity for the treatment of falciparum malaria (Shanks, 2006). Artemisinin resistance was first reported in Southeast Asia in 2008, and evidence suggests that it is spreading (Ashley, 2014; Dondorp, 2009; Thriemer, 2014). Widespread resistance to the most powerful new weapon in the fight against malaria could de-rail many ongoing efforts to eradicate the disease in the endemic world. As such, it is widely acknowledged that combination therapy should be the standard of care. The WHO currently recommends artemisinin combination therapy (ACT) consisting of DHA-piperaquine, followed with a 0.75 mg kg^{-1} single dose of primaquine for the treatment of uncomplicated malaria (WHO, 2010).

At present, research is still ongoing as to the exact killing mechanism for the functional endoperoxide bridge contained in the artemisinins, however, it is thought to be a result of membrane depolarization and subsequent interference with electron

transport (Antoine, 2014). Further development of synthetic and semi-synthetic artemisinins is still ongoing as well as the development of novel endoperoxide containing compounds which have proven effective as anti-malarial agents in pre-clinical studies (Lanteri, 2014; Oliveira, 2014).

3.4.4.4 Repurposed Drugs

For the treatment of malaria and many other tropical diseases, limitations in funding have led to discoveries in the area of repurposing of drugs commonly used for other indications. Broad spectrum antibiotics have proven highly successful in the treatment of malaria. Doxycycline, a synthetic tetracycline, was shown to have partial prophylactic efficacy against malaria in the 1970's (Andrews, 2014). In fact, despite its own set of tolerance issues, doxycycline is now the prophylactic drug of choice for the US Army due to more serious concerns with mefloquine (Kime, 2012). It is also effective for use in treatment when partnered with a fast acting anti-malarial like quinine or quinidine (Tan & Centers for Disease Control and Prevention, 2011). Other antibiotics including clindamycin and sulfonamide antibiotics like sulphadiazine and sulphadoxine have also proven effective in the treatment of malaria (Andrews, 2014). Many of these target folate synthesis, which is a common pathway for other classical anti-malarials. Antifolate drugs block the synthesis of tetrahydrofolate, which in turn shuts down nucleic acid synthesis (Shanks, 2006). Among these is dapsone. Dapsone, a sulfone, was first used to treat leprosy but was introduced by GSK as Lapdap (dapsone/chloroproguanil) in the late 1990s. This drug was removed from the market in 2008 due to hemolytic anemia similar to that of the 8-aminoquinolines. Exploration of this strategy still continues with significant effort. Several examples can be found in the literature with protease inhibitors, antibiotics, and quinolones found to act against various targets in the parasite (Rosenthal, 2003). With pre-existing and well established safety profiles, re-purposing of old drugs is an attractive strategy if for no other reason than bypassing several regulatory hurdles and saving money in development. Another potential benefit for the developing world is often the cost of these drugs once marketed. Doxycycline, for example, is pennies per dose compared to Malarone (atovaquone/proguanil) and inexpensive drugs are far more likely in that regard to make a meaningful impact on the fight against parasitic disease worldwide.

3.4.5 Natural Products in the Treatment of Malaria

As we have noted many of the progenitive compounds (quinine, artemisinin) for the treatment of malaria are naturally derived. Efforts continue to isolate natural compounds with anti-protozoal activity (Mohammed, 2014; Singh, 2014; Traore,

2014). Natural sources including plants, microbes, and animals provide libraries with rich chemical diversity, however, isolation and purification of active components presents a significant challenge in natural product development. The complexity of isolation is highly dependent on structure, which can often be quite complex with compounds isolated from natural sources. This of course leads to a second challenge for the development of natural products, namely synthesis. Once a hit is identified from a mixture, large quantities are often needed for further pre-clinical or clinical testing. The likelihood of identifying a clinic-ready compound from a natural source in today's regulatory environment is small. The more likely scenario involves the identification of a hit compound, which can be potentially modified to improve drug-like characteristics or decrease potential liabilities from toxicity. As we will discuss in the next section, the source of starting material for a drug development project is largely irrelevant once a hit is identified, as all compounds should be gated through the same testing scheme to ensure the end product matches the target product profile.

3.4.6 Considerations for Anti-parasitic Drug Development

In any drug candidate's journey down a development pipeline there are many pitfalls. Sadly, activity against a biological target or even *in vivo* efficacy does not make a successful drug. Careful consideration should be given early to liabilities that might lead to late stage failure, which could be costly and drain resources vital to the discovery of a potential candidate. In 1991, the leading cause of attrition for drug candidates was poor pharmacokinetics and/or bioavailability (40%), yet by 2000 this had decreased to less than 10% (Kola & Landis, 2004). This is largely attributable to the advent of *in vitro* screens for physiochemical properties likely to create downstream liabilities. The placement of these screens early in the pipeline forces compounds with poor solubility, bioavailability, and metabolic stability to fail during far cheaper stages of development and spares the opportunity cost otherwise missed in development of these compounds. Fig. 3.4.6 outlines a generic paradigm for screening compounds. In this paradigm early hits from *in vitro* activity screens are passed through an absorption, distribution, metabolism, and excretion (ADME) gate before passing into more expensive animal models. The source of compounds could be from commercially available large libraries, libraries of natural product extracts, small scale synthesis, etc. The process remains unchanged. However, feedback from all stages should be iteratively incorporated into the next round of screening. Acceptable physiochemical properties for a drug ultimately depend on the target product profile for intended use; however, it is reasonable to assume that compounds with low solubility or poor permeability may not fit a profile for oral prophylaxis, for example. It should also be noted that many of these properties go hand-in-hand, and that it is often necessary to strike a balance. For example, lipophilicity, permeability, and metabolic instability tend to correlate since CYPs tend to favor lipophilic drugs.

From this example, if one were trying to develop a long half-life lipophilic drug to concentrate in the food vacuole of a parasite, it might be necessary to trade off some lipophilicity (and with it potentially some biological activity) to enhance half-life. The subtleties of this interplay of course depend again on the intended use of the drug. Feedback from all stages of screening should be used to inform later rounds. In such a manner, successive rounds of screening should get closer and closer to the desired chemical space with optimum physiochemical properties and biological activity for intended use.

From the example of the 8-aminoquinolines, a very modern consideration for drug development arises. Pro-drugs should be fully metabolically characterized, with all pertinent pathways identified. Consideration should be given to the pharmacogenomic make-up of the target population. Pro-drugs activated by CYPs 3A4, 2D6, or 2C19 may lack efficacy in some populations due to high polymorphism. If at all possible, non-CYP mediated conversion is desirable to avoid such liabilities in later development. Likewise, toxicity can be affected in a similar manner if, for example, the formation of a reactive metabolite is mediated by a highly polymorphic CYP.

Table 3.4.1: Approaches to antimalarial drug discovery and development. Adapted from Philip J. Rosenthal's review on Antimalarial drug discovery: old and new approaches (Rosenthal, 2003).

Approach	Examples
Optimize therapy with existing drugs (combinations)	Amodiaquine/sulfadoxine/pyrimethamine Amodiaquine/artesunate Artesunate/sulfadoxine/pyrimethamine Artesunate/mefloquine Artemether/lumefantrine Chlorproguanil/dapsone Chlorproguanil/dapsone/artesunate Atovaquone/proguanil
Develop analogs of existing drugs	New aminoquinolines New endoperoxides New folate antagonists
Natural Products	New natural products
Repurposing of drugs from other therapeutic areas	Folate antagonists Antibiotics Atovaquone Iron chelators
Reversing drug resistance	Verapamil, desipramine, trifluoperazine, chlorpheniramine
Discovery of compounds active against novel targets	Antibiotics, Quinolones, etc.

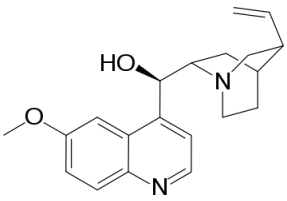

Figure 3.4.1: The structure of quinine.

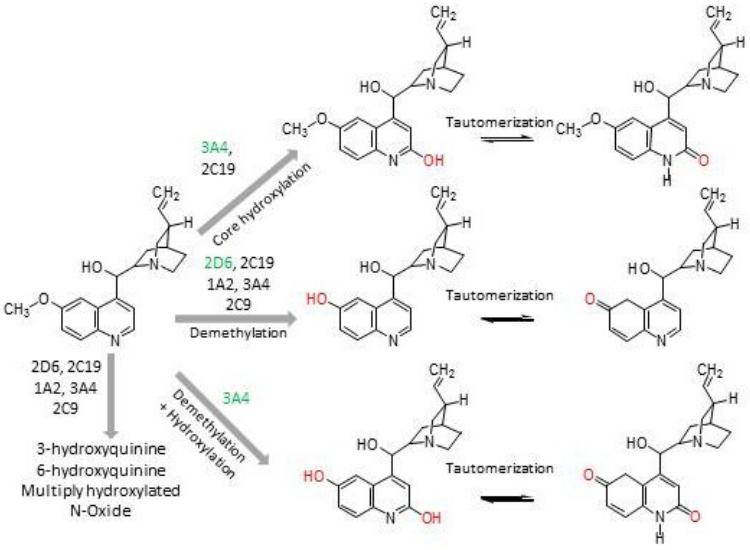

Figure 3.4.2: Metabolism of quinine leads to the formation of redox active quinones. Adapted from Marcsisin 2013 (Marcsisin, 2013).

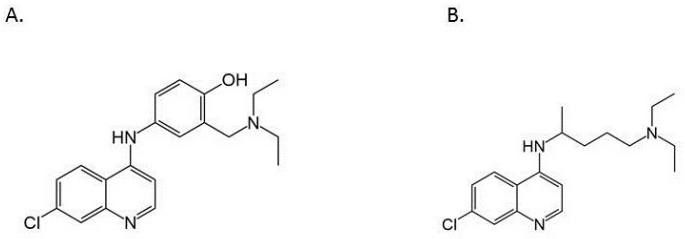

Figure 3.4.3: Two important members of the 4-aminoquinoline class: A. Amodiaquine B. Chloroquine

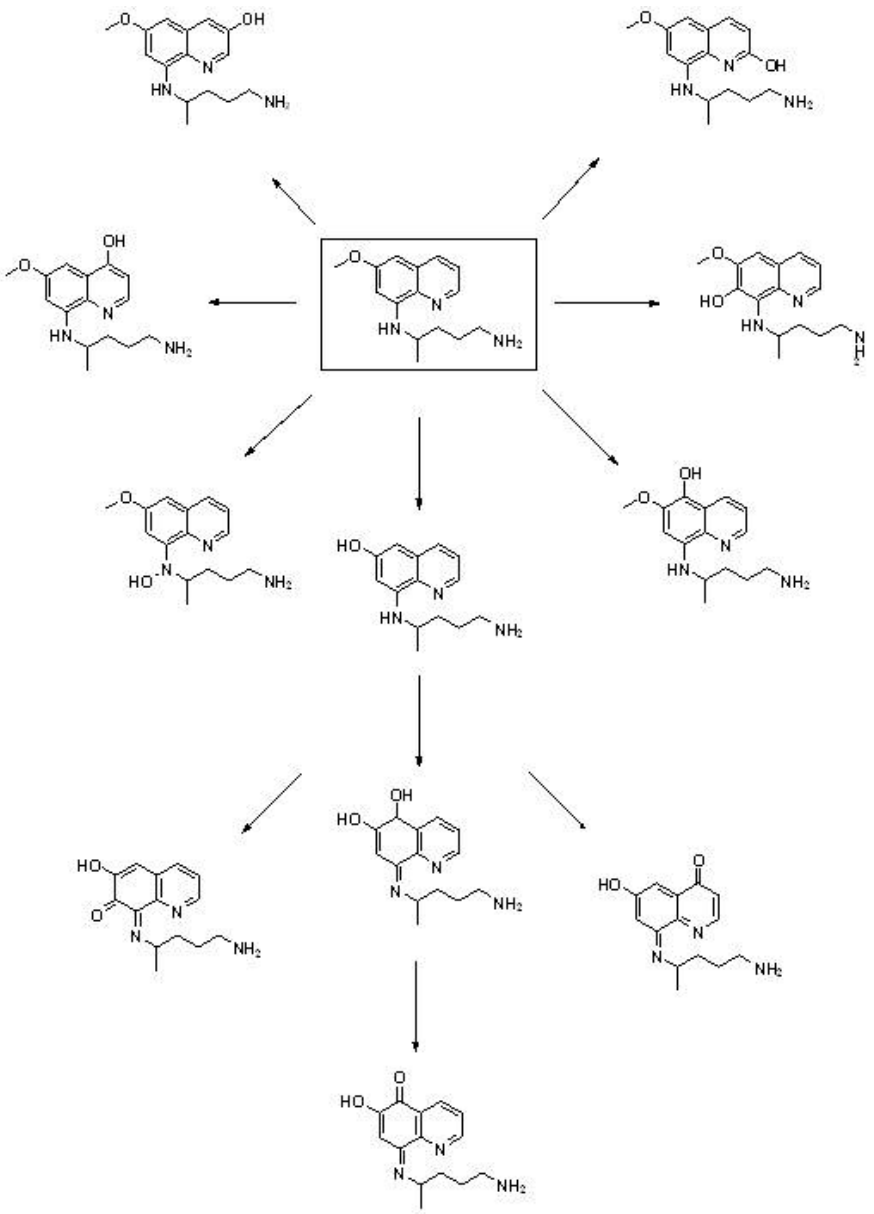

Figure 3.4.4: CYP 2D6 mediated metabolism of primaquine (Pybus, 2013). Several potentially oxidative metabolites formed by CYP 2D6 may be key in primaquine activity. Carboxyprimaquine is the primary metabolite found is plasma. Its production is likely MAO mediated and the pathway is not shown.

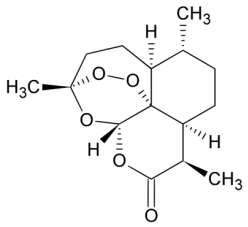

Figure 3.4.5: Artemisinin was identified as the active ingredient in sweet wormwood in the 1970s. Several synthetic artemisinins and other endoperoxides now exist for the treatment of malaria.

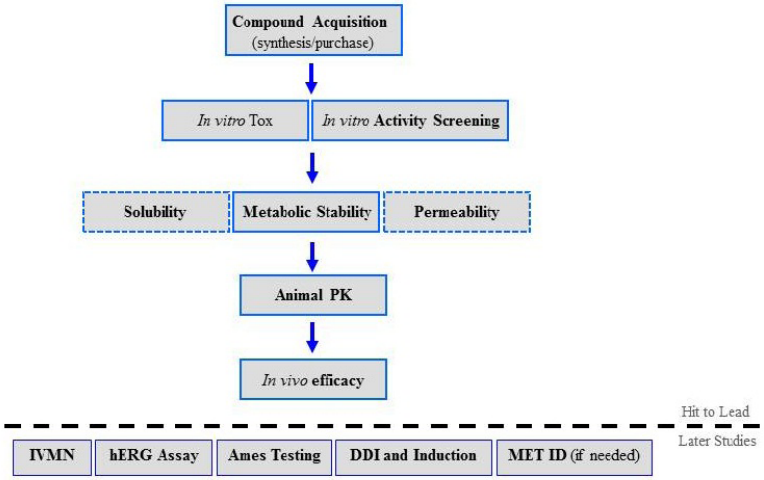

Figure 3.4.6: Generic paradigm for drug discovery that uses ADME properties to down-select potential candidates prior to expensive and labor intensive animal modeling. Dashed lines indicate a screen that is not necessarily a mandatory gate, but which can aid in the interpretation of potential later stage failure.

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