WALTER CABRI
FRESENIUS KABI ANTI-INFECTIVES
CERNUSCO SUL NAVIGLIO (MI)
WALTER.CABRI@FRESENIUS-KABI.COM

DOI: HTTP://DX.MEDRA.ORG/10.17374/CI.2016.98.4.48

FIGHT AGAINST ONCHOCERCIASIS/LYMPHATIC FILARIARIS AND MALARIA: AVERMECTIN AND ARTEMISININ, LEAD COMPOUNDS FROM NATURE

The fight against onchocerciasis/lymphatic filariaris and malaria has been centered on the use of natural products, avermectin/ivermectin and dihydroartemisinin/artemisinin respectively. The 2015 Nobel Prize in Medicine and Physiology given to Satoshi Omura/William C. Campbell for ivermectin and Youyou Tu for artemisinin represents an important recognition of the efforts of scientists in the war against tropical diseases. The drug discovery and development process was very complex and required money and top quality scientists and specialists in several areas.

Ge Hong (283-343) described the use of Artemisia Annua in his *Handbook of Prescriptions for Emergencies*

In the XIX and XX centuries with the progress of science, the fight against viruses, bacterial infections and parasites was at the top of the agenda of scientists. With a few exceptions, three microbiological schools, the French, the German and the English one, were competing and leading the research at the beginning of the last century. Pharmaceutical science made extraordinary discoveries during the last 100 years; however, nature always reacted to the introduction of new drugs and treatments. In fact, we are still working nowadays to find cure for new viruses, resistant bacteria, and unfortunately, the fight against tropical parasites is still on.

In the history of tropical diseases, natural products coming from fermentation or plant extraction have played a key role in the discovery of new medicines: tetracyclines and avermectin for onchocerciasis/lymphatic filariaris, quinine and artemisinin for malaria, amphotericin B and paramomycin for leishmaniosis, rifampicin for leprosy. Even if very successful in the past, drug discovery programs based on natural products are not anymore in line with the new trends of big pharma companies. Nevertheless, natural products are still important for the identification of new biological targets in drug discovery (i.e. rapamycin/mTOR, sphingoside/sphingosine kinases, the endocannabinoid system, etc.). When not based on an educated guess or ethno medicine, research on natural products requires an opposite approach respect to current drug discovery process. Instead of designing a molecule for a specific target, you have a molecule that is looking for an application, for a biological target, for a disease. In addition, most of the times the complex structure of natural products does not allow generating large libraries to feed the biological screening. Natural products, like avermectin and artemisinin, allow a very limited manipulation [1].

Onchocerciasis

In 1874, John O'Neill, an Irish surgeon in the British Navy that was working at Addah Fort (Ada Foa) Hospital in the Gold Coast (Ghana), discovered filarial parasitic infections. A few years later in 1890, Rudolf Leuckart (German) and Sir Patrick Manson (British and founder in 1899 of The London School of Hygiene & Tropical Medicine) isolated and identify adult worms responsible for onchocerciasis. The other important scientists involved in the identification of the disease were the French Jean Montpellier/Adrien Lacroix and Emile Brumpt and Rodolfo Robles from Guatemala.

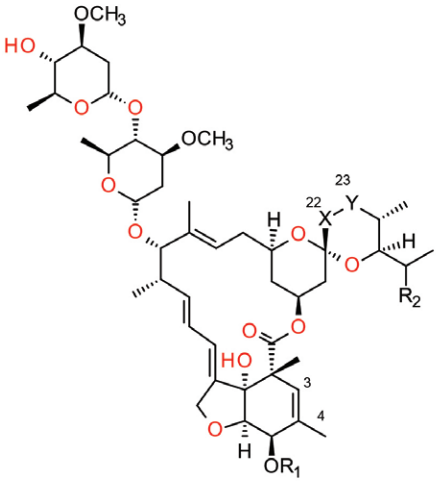
Avermectin discovery and development

In 1970, after a successful 33 years career in Merck Sharp and Dohme, Max Tisher moved to Wesleyan University. Satoshi Omura (2015 Nobel Prize in Physiology and Medicine) spent a sabbatical in Tisher lab during 1971. Omura was coming from the prestigious Kitasato Institute founded in 1914 by Shibasaburo Kitasato, a Robert Kock fellow that played an important role in the development of a serum therapy for tetanus and antitoxins for diphtheria and anthrax. In the context of an international collaboration between Academia and Industry and thanks to the Omura-Tisher link, in 1973 KI and MSD decided to start a collaboration for the discovery of veterinary drugs with anthelmintic activity under the leadership of William C. Campbell (2015 Nobel Prize in Physiology and Medicine) [2].

The Japanese team identified *Streptomyces* species from several sources and carried out an *in vitro* test. However, the screening was difficult to be evaluated because of the many toxic secondary metabolites produced by *Streptomyces*. In 1973 the most promising 53 microbial samples were shipped to MSDL and the fermented broths were tested by an innovative *in vivo* experiment, a mice infected with nematode worm *Nematospiroides dubius*. The Kitasato team was focused on *Streptomyces* species because these microorganisms have been crucial for the drug discovery of several pharmaceutically active compounds. In fact, *Streptomyces species* are able to generate a great variety of secondary metabolites (i.e. streptomycin (1944), cephalosporins (1945), chloramphenicol (1949), neomycin (1949), tetracycline (1950), erythromycin (1952), paramomycin (1950s), adriamycin (1950s), nystatin (1950), amphotericin b (1953), spectinomycin (1961), lincomycin (1962), oleandomycin (1962), gentamicin (1963), fosfomycin (1966), geldanamycin (1970), daptomycin (late 1980s), tetracyclin (1948), clavulanic acid (1974), rapamycin (1975), and etc.) [3].

The microorganism OS-3153 gave the most promising results, in spite of the very high toxicity for the host, in fact, very active toxic secondary metabolites were present in the fermented broth. MSDL improved the fermentation process and found a better therapeutic window. The culture was renamed MS-4680 and the active fraction named C-076. The microorganism was later classified as a new species of actinomycete, *Streptomyces avermectilis* [4]. The biological activity triggered the research program to identify the lead compound. Miller team in MDSL isolated and characterized the 8 members that belong to the 16 lactone ring avermectin family, A_{1a}, A_{1b}, A_{2a}, A_{2b}, B_{1a}, B_{1b}, B_{2a}, B_{2b}. Merck disclosed in 1979 with three papers on antimicrobial agents and chemotherapy the avermectins product and their biological activity [5] (Fig. 1). It was observed that Avermectin B1 series was more potent than the B2 one orally, while the converse was true with a parenteral administration.

Therefore, the efforts of MSD scientists was devoted to the selective hydrogenation of the C22-23 double bond to get the best potency achieving oral availability. In 1977 Chabala and Fisher filed a patent on the selective C22-23 double bond hydrogenation catalyzed by the Wilkinson catalyst of the avermectins B_{1a} and B_{1b} in 80/20 mixture [6]. The research was published on the *Journal of Medicinal Chemistry* in 1980 and one year later the 80/20 mixture of Ivermectins B_{1a} and B_{1b} with the trademark of Ivomec® was launched as a broad spectrum anti-parasitic veterinary drug. Since 1977, Campbell team extended the pharmacological studies to the human parasite and already in 1982 the first clinical trials started in collaboration with the WHO. MSD was able to get a profitable business with Ivomec® in the veterinary segment and gave Mectizan® for free the drug to the WHO for the fights against tropical diseases [7]. Mectizan® was approved in 1987 for onchocerciasis and in 1998 in combination with albendazole, donated by GSK, for lymphatic filariaris.



	R1	R2	X-Y
Avermectin A_{1a}	CH_3	CH_2H_3	CH=CH
Avermectin A_{1b}	CH_3	CH_3	CH=CH
Avermectin A_{2a}	CH_3	CH_2H_3	$CH_2CH(\alpha OH)$
Avermectin A_{2b}	CH_3	CH_3	$CH_2CH(\alpha OH)$
Avermectin B_{1a}	H	CH_2H_3	CH=CH
Avermectin B_{1b}	H	CH	CH=CH
Avermectin B_{2a}	H	CH_2H_3	$CH_2CH(\alpha OH)$
Avermectin B_{2b}	H	CH_3	$CH_2CH(\alpha OH)$
Ivermectin B_{1a}	H	CH_2H_3	CH_2CH_2
Ivermectin B_{1b}	H	CH	CH_2CH_2

Fig. 1

More than 1 billion treatments have been approved from 1987 to 2012 in 25 years of Mectizan Development Program. The drug was used in 28 countries in Africa and 4 in South America. Colombia, Ecuador, Mexico and the Sudanese Region have been declared free from oncocherchiasis. Since 2000 more than 665 million treatment Mectizan®/albendazole combination therapies have been approved in Yemen and 28 African countries for the fight against lymphatic filiarisis. The number of treatments increases every year and now the Lymphatic Filiarisis Elimination (LFE) program has been launched.

Malaria

In the field of tropical diseases the Italian School played a major role in the research against malaria. Alphonse Laveran (French), Donald Ross (1902 Nobel Prize in Physiology and Medicine) and Patrick Manson (both British) were involved in the malaria research with the Italian School that comprised Stefano Golgi (1906 Nobel Prize for Physiology and Medicine), Ettore Macchiafava, Angelo Celli, Giovanni Battista Grassi, Amico Bignami, and Giuseppe Bastianelli. Italy was deeply involved in the research not because of the colonies but because in Italy malaria was endemic [8].

Artemisinin discovery and development

Malaria is the only disease with an Italian name malaria (bad air) and it is caused by a parasite *Plasmodium* that is spread by a mosquito, the

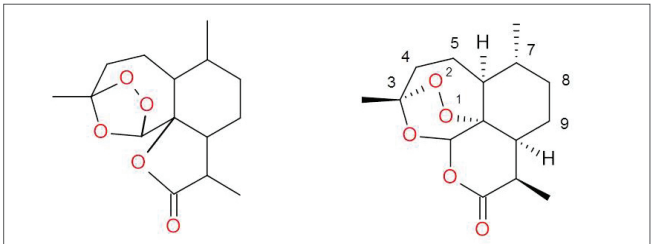


Fig. 2

Anopheles. There are five species of *Plasmodium* and the most dangerous one, affecting mainly Africa and Asian countries, is the *Falciparum*. The impact of natural products in the fight against malaria has been extraordinary. The use of quinine, first isolated by Pelletier and Caventou, in combination with other initiatives devoted to the elimination of the mosquito, allowed the eradication of the disease in Europe and the containment in other countries. Nowadays, artemisinin-based combination therapies are the most important tools in the process for the eradication of *Plasmodium falciparum* in endemic countries [9].

Artemisinin was first isolated in 1972 from an Artemisia Annua coming from the surroundings of Beograd by a Yugoslavian chemist Milutin Stefanovic. He characterized the product using a 60 MHz NMR and unfortunately he described in a poster at the 8th International Congress on the Chemistry of Natural Products (New Delhi February 1972) a wrong structure (Fig. 2) [10]. Stefanovic was a simple chemist devoted to the isolation of new chemical entities from plants and his research did not have any relationship with malaria. Meanwhile, Project 523 started on May 23rd 1967 and involved more than 500 scientists in about 60 different laboratories and institutes all over China. The main target of Project 523 was the identification of new antimalarial medicines to help Vietnam in the war against USA. In fact, during the Vietnam War a *Plasmodium Falciparum* resistant to chloroquine was killing soldiers of both parties more than bullets. For this reason in 1967, the North Vietnam leader Ho Chi Minh called Chinese President Mao Zedong asking from help. China was in the middle of a political turmoil, the Cultural Revolution. In spite of that, Project 523 started in secret and delivered three treatments by 1969. However, the long-term goal was the development of a potent antimalarial drug by screening the knowledge of the ancient Chinese medicine [11]. Only several years later it was clear that Youyou Tu (2015 Nobel Prize in Physiology and Medicine) gave the most important contribution to the discovery of artemisinin. She was a principal investigator of Chinese Materia Medica, Chinese Academy of Chinese Medical Sciences [12]. During the Nobel Prize lecture, Youyou Tu reported November 1972 as the date for the isolation of artemisinin. More than 200 recipes and 380 extracts from herbs were tested in a rodent model. The more potent was the extract of Qinghao (the Chinese name of *Artemisia Annua*). The discovery was coming from the work of Ge Hong, a pharmacist/alchemist (283–343) that described the use of Qinghao for alleviating intermitted fever (malaria). Ge Hong described also the extraction process in *A Handbook of Prescriptions for Emergencies*: "A handful of Qinghao immersed with 2 liters of water, wring out the juice and drink it all". A very mild extraction process that prevents the decomposition of the peroxide fragment of artemisinin. The mode of action of artemisinin derivatives is still undefined; however, the presence of the hydroperoxide fragment is critical to get antimalarial activity. Youyou Tu used cold ethanol instead of cold water to get reproducible data using the extract. Only in 1980, the Chinese team described the X-ray of artemisinin and solved the structure [13]. Two key clinical studies were carried out in 1982 and 1984 in China. Later the same team discovered dihydroartemisinin, artesunate and artemeter. In China the use of artemisinin compounds as monotherapy became the standard, generating some issues. In fact, artemisinin resistance has been verified in Greater Mekong river basins. The main issue was the short half-life of artemisinin: the treatment was very effective and patients recovered rapidly, but most of them did not complete the treatment allowing reinfection to occur. This was a clear way to generate resistance. For this reason WHO strongly discourage the use of artemisinin derivatives monotherapies.

Only with the arrival of big pharma (Novartis, Sanofi and Sigma-tau) and Foundations (i.e. Malaria Medicine Venture) under the guidance of WHO, Artemisinin Combination Therapies (ACTs) have been developed and introduced for the treatment in endemic countries. During recent years three

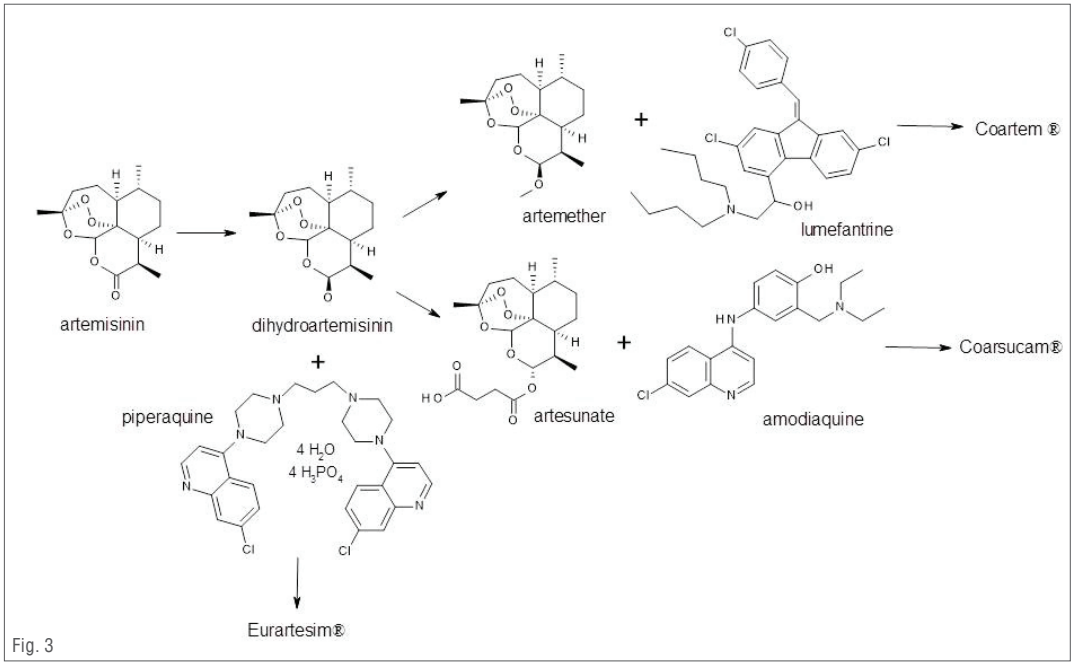
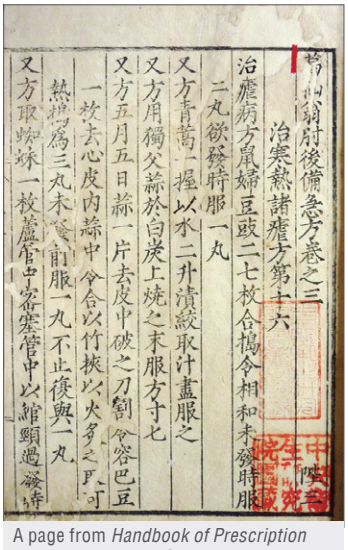


Fig. 3



A page from *Handbook of Prescription for Emergencies* by Ge Hong

potent ACTs have been registered and produced under cGMP for the treatment of malaria by Novartis (artemether-lumefantrine, Coartem®), Sanofi-Aventis (artesunate-amodiaquine, Coarsucam®) and Sigma-tau-MMV (dihidroartemisinin-piperaquine, Eurartesim®). ACTs are based on the combination of an artemisinin derivative with a very short half-life and a synthetic antimalarial medicine characterized by a longer half-life [Lumefantrine ($t_{½}$ 3-6 days), Amodiaquine ($t_{½}$ 9 days) and Piperaquine ($t_{½}$ 22 days)] in order to protect from rapid reinfections after artemisinin derivative disappearance. Interestingly, the international community was able to generate reliable and comprehensive specifications and analytical methods for artemisinin more than 30 year after its structural identification. The critical isolation/identification of artemisinin impurities and the determination of the relative response factor (RRF) was achieved in 2011 by a joint effort of Sigma-tau and Bill Clinton Foundation [14].

More than three billions people are at risk of malaria and this led to about 214 million malaria cases in 2015 (uncertainty range 149 million to 303 million) and estimated 438000 deaths (uncertainty range 236000 to 635000). Malaria deaths in children under 5 years, mainly in Africa, remain the most important humanitarian emergency, 306000 death in 2015 (range: 219000-421000). These numbers are still impressive; however, mainly because of the introduction of ACTs, between 2000 and 2015, malaria incidence among populations at risk fell by 37%. In that same period, malaria death rates among populations at risk fell by 60% globally among all age groups and by 65% among children under five [15].

Mode of action of ACTs is still under investigation [16]. The unstable nature of products like dihydroartemisinin does not help, in fact, as soon as the molecule gets in contact with water and acidic media a mixture of structures with antimalarial activity is generated [17]. It is worth noting that once the drug is absorbed in the intestine it can directly attack the parasites into the red blood cells. The short half life is not a negative characteristic, in fact, in our opinion it allows to limit other negative effect related to the formation of oxygen centered electrophilic radicals [18]. Trioxolane with longer half life like arterolane have the time to be distributed in the body potentially increasing the long term toxicity [19].

Among tropical diseases, malaria and onchocerciasis/lymphatic filariaris, thanks to the money of international organization, the involvement of WHO and the efforts of expert scientists of large pharmaceutical companies, are not anymore neglected. Potent, safe and effective drugs are now available. The real key for the eradication of these diseases is the distribution of the drugs and for malaria the elimination/control of the vector.

We hope that a similar approach will be replicated by the international community to fight other dangerous neglected tropical diseases knowing that poverty is the other serious threat.

BIBLIOGRAFIA

[1] J.W.-H Li, J.C. Vederas *Science*, 2009, **325**, 161.
[2] W.C. Campbell, *Med. Chem. Rev.*, 1993, **13**, 61.
[3] a) J. Mann, Life saving drugs: The Elusive Magic Bullett Chapter 2, p.70, RCS paperbacks, Cambridge, 2004; b) R.E. de Lima Procópioa *et al., Braz. J. Infect. Dis.*, 2012, **16**, 466.
[4] O. Satoshi, A. Crump, *Nature Reviews Microbiology*, 2004, **2**, 984.
[5] a) R.W. Burg *et al., Antimicrob. Agents Chemother.*, 1979, **15**, 361; b) T.W. Miller *et al., Antimicrob. Agents Chemother.*, 1979, **15**, 368; c) J.R. Egerton *et al., Antimicrob. Agents Chemother.*, 1979, **15**, 372.
[6] a) J.C. Chabala *et al., J. Med. Chem.*, 1980, **23**, 1134; b) J.C. Chabala, M.H. Fisher, US 4199569 (Priority 3 October 1977).
[7] G. Burnham, T. Mebrahtu, *Tropical Medicine and International Health*, 2004, **9**, a26.
[8] F. Snowden, The Conquest of Malaria: Italy, 1900-1962, Yale University Press, New Haven, 2005.
[9] S.R. Meshnick, M.J. Dobson, The History of Antimalarial Drugs in Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery, chapter 2 p 15, P.J. Rosenthal (Ed.), Humana Press Inc., Totowa, NJ, 2001.
[10] D. Jeremic *et al.*, 8th International Congress on the Chemistry of Natural Products abstract, 1972, **C-57**, 222.
[11] L.H. Miller, X. Su, *Cell.*, 2011, **146**, 855.
[12] Y. Tu, *Malaria Nature Medicine*, 2011, **17**, 1217.
[13] Qinghaosu Research Group, *Scientia Sinica*, 1980, **23**, 380.
[14] R.W. Stringham *et al., J.Chromatography A*, 2011, **1218**, 6838.
[15] World malaria report 2015 by WHO, http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/
[16] a) S.R. Mesnick, *Int. J. Parasitol.*, 2002, **32**, 1655; b) R.K. Haynes *et al., Infect. Disord. Drug Targets* 2013, **13**, 217.
[17] W. Cabri, F. Giorgi, F. Gasparrini, unpublished results.
[18] C. Pisano, L. Vesci, US9023861(filing 12.6.2010), DNA damage caused by radical species in connection with PARP inhibitors triggered apoptosis.
[19] I.M. Copple *et al., Mol. Med.*, 2012, **1**, 1045.

Lotta contro oncocercosi/filariosi linfatica e malaria: ivermectina e artemisinina sostanze-chiave dalla natura

La lotta contro malattie tropicali come oncocercosi/filariasi linfatiche e malaria è in questo momento basata su farmaci derivati da sostanze organiche naturali (avermectina/ivermectina e artemisinina/artemisinin combination therapies, ACTs). I premi Nobel per la Medicina 2015 sono stati conferiti a Satoshi Omura/William C. Campbell per l'ivermectina e a Youyou Tu per l'artemisinina, come riconoscimento degli sforzi che questi scienziati hanno profuso nella lotta contro le malattie tropicali. La lotta contro le malattie tropicali è del tutto analoga a quella di qualsiasi malattie con l'unica differenza del numero di soggetti da trattare e richiede tempo, denaro e l'usuale elevato livello scientifico necessario per lo sviluppo di qualsiasi altro farmaco.