THE PRESENT STATUS OF MALARIA CHEMOTHERAPY[1]

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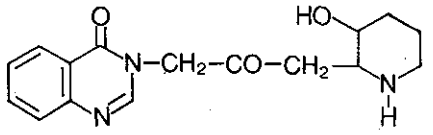
This paper is dedicated to Dr. Ken'ichi Takeda, Research Director of Shionogi and Co., Ltd., Osaka, on the occasion of his 70th birthday.

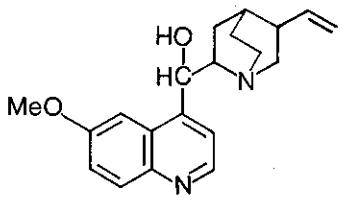
The present status of malaria chemotherapy is reviewed.

Those peoples exposed to malaria over centuries long ago learned that extracts of certain natural products had beneficial or even curative effects on this epidemic disease. The powdered root from a Chinese plant containing febrifugine as the active agent and preparations from the bark of the Cinchona trees containing quinine have been used long before their active principles were isolated in pure form (1).

Natural Products as Antimalarials



Febrifugine



Quinine

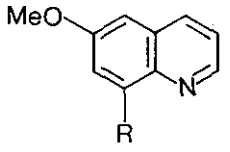
This paper was distributed at the First Meeting of the WHO-Task Force on the Chemotherapy of Malaria, Geneva, November 24-28, 1975.

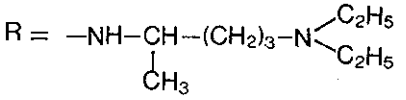
The structure of febrifugine was established in 1950. It is a powerful emetic agent, and has, therefore, never found wide clinical use. The story regarding the active ingredients of Cinchona preparations, introduced in crude forms since 1677, is quite a different one, despite the fact that it took more than 150 years before Pelletier et al. isolated the active principles and nearly another 100 years before their structure was established.

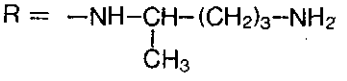
Empirical Approaches :

Since the enemy controlled the quinine supplies during World War I, German scientists had to develop new antimalarials. The knowledge that quinine contains a quinoline moiety together with the finding of Ehrlich that the dye-stuff methylene blue possesses some antimalarial activity, stimulated interest in quinoline and acridine derivatives with basic side chains.

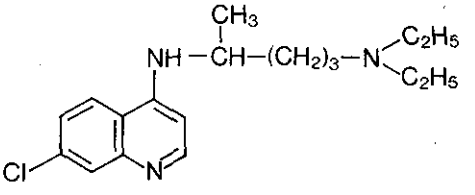
8-Aminoquinolines



R =  Plasmochin®, Pamaquine

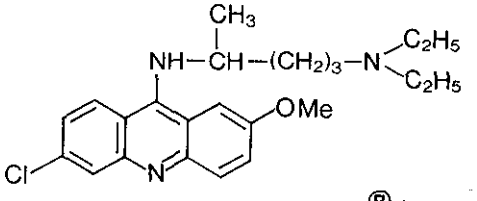
R =  Primaquine

4-Aminoquinolines



Chloroquine

Acridines



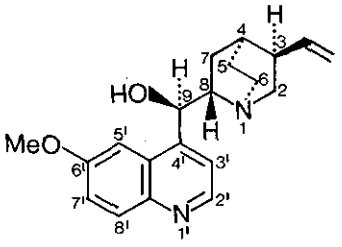
Quinacrine, Atebrin®

The clinical results obtained with pamaquine and primaquine as the best candidates among the 8-aminoquinolines, and chloroquine and quinacrine, as the most promising compounds in the 4-aminoquinoline and 9-aminoacridine series, contributed to a feeling that the problem of malaria was solved. The clinicians had a variety of effective drugs for the treatment of all stages of this disease. This expectation has, however, been clouded by the emergence of strains of plasmodium species resistant to those drugs. Furthermore, toxicity, photosensitivity, staining of the skin and other side effects resulted in the recognition that more work had to be done before the ideal antimalarial drug could be claimed.

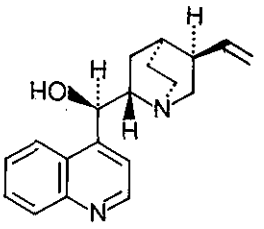
Directed Approaches to the Development of New Antimalarials :

From the bark of Cinchona trees some twenty alkaloids have been isolated. From the four major alkaloids present, only quinine and cinchonidine have found widespread clinical use as antimalarials and this mainly for economical reasons: up to 10% of quinine is present in certain Cinchona species, whereas its isomers quinidine and cinchonine are contained in the natural

material only in much smaller amounts. Quinine has become the natural antimalarial of choice and has still retained its position.



Quinine



Cinchonidine

The chemical structures of quinine and cinchonidine differ from each other by the presence of a methoxy group at C-6' in the former and are quite complicated. They both contain four asymmetric carbon atoms, three present in the quinuclidine moiety, and one in the hydroxymethylene bridge combining the quinuclidine with the quinoline moiety. This gives rise in each series to sixteen optically active isomers. Eight of these isomers have the various substituents at the asymmetric carbon atoms in a different relative position to each other, and the other eight are represented by their mirror-images. Quinine is one of these sixteen optical isomers. This knowledge, elaborated by Rabe and collaborators, who synthesized dihydroquinine in 1931 (2),

and the work of Prelog and others, who established the absolute configuration of quinine (3), was proven correct by a total synthesis of natural quinine, accomplished by Woodward and Doering in 1944 (4). Whereas the total synthesis of quinine by Woodward et al. can be marked as a milestone in natural products chemistry, it is not economical. It even had little importance for the finding of a "better" quinine.

Medicinal chemists have learned since then that the relative and absolute configurations of drugs containing asymmetric functions are of essential importance for biological activity.

We considered it attractive to improve the total synthesis of quinine, and to prepare at the same time analogs in the quinine and quinidine series with different aromatic substituents, a challenge which had never been attempted before. This project was greatly facilitated by prior clinical and experimental results in this field. The following was common knowledge:

1) Quinine seemed more active against certain malaria parasites than cinchonidine, the other major alkaloid.

2) Dihydroquinine, a minor alkaloid, seemed to be as active as quinine.

3) Replacement of the methoxy group in quinine by a hydroxy group or other ether groups had little effect on the antimalarial activity.

4) The C-8/N-1 bond in the quinine molecule seemed to be essential.

5) The elimination of the hydroxy group at C-9 resulted in a complete loss of activity.

6) The major metabolite of quinine in man, the 2'-hydroxy-derivative, was much less active than the parent drug.

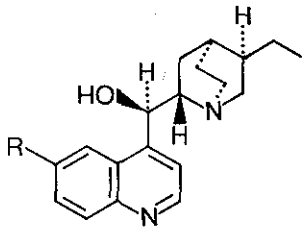
With these findings, together with much improved know-how in chemical methodology, a program was established in 1968 at Hoffmann-La Roche Inc., Nutley, under the leadership of Dr. Uskokovic and a group of able collaborators, to answer the following questions:

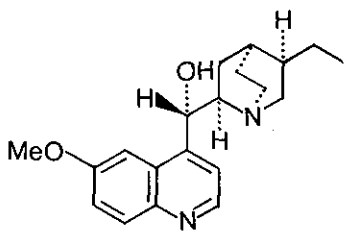
1) Are dihydroquinines or dihydroquinidines, which both are much easier to synthesize, as active as their corresponding analogs having a vinyl group at C-3?

2) Is it necessary to prepare optically active quinines and quinidines, or would racemates, which are much easier to obtain by total synthesis, do equally well?

3) Which is the optimal substitution pattern in the quinoline part of Cinchona alkaloid analogs?

First we prepared by improved synthetic methods racemic, natural and unnatural dihydroquinine. We found that all three compounds were equally active and had the same toxicity in mice infected with Plasmodium berghei[5]. Equal activity and toxicity were also seen in racemic and natural 6-chloro-dihydrocinchonidine, where the methoxy group in position 6' of dihydroquinine was replaced.





R = CH_3O: Dihydroquinine

R = Cl: 6-Chloro-dihydro cinchonidine

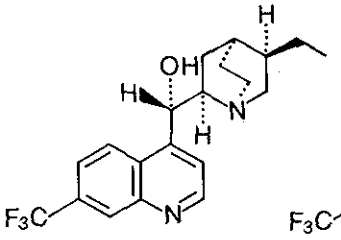
Dihydroquinidine

The dihydroquinidine isomers were found to be even more active against Plasmodium berghei under the same experimental conditions. Their antimalarial activities, when tested in form of the dihydrochlorides, were found superior to that of quinine in Prof. Brener's test system.

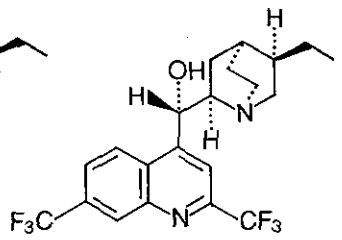
These interesting experimental results showed for the first time that it may not be necessary to use optically active quinine derivatives. The second important finding, already observed much earlier, established that dihydro derivatives are just as active as their parent compounds. This is a great comfort to the organic chemist, since the introduction of an ethyl group is much easier than the introduction of a vinyl unit. To answer the question regarding the optimal aromatic substitution pattern was more difficult, since every compound had to be prepared from new starting materials.

With the knowledge that racemic dihydro compounds would give a meaningful answer, dozens of analogs were prepared, out of which Ro 20-5331, Ro 21-0960 and Ro 21-3473 emerged as the most interesting candidates. They were compared with quinine and mefloquine. The latter is a synthetic compound evaluated by the Walter Reed Army

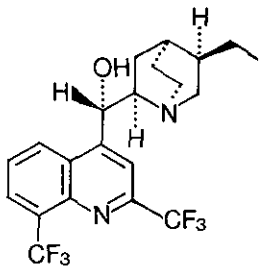
Institute of Research (WRAIR) and found to be an exciting new antimalarial, more active than quinine, even in quinine and chloroquine resistant strains. Mefloquine is under clinical investigation (6).



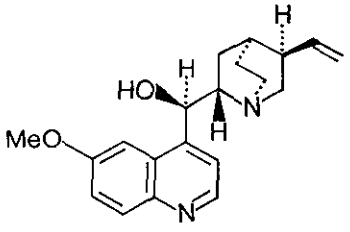
(±) Ro 20-5331



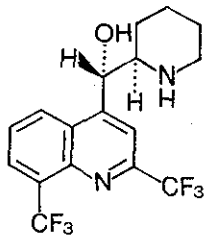
(±) Ro 21-3473



(±) Ro 21-0960



(-) Quinine



(±) Mefloquine

Whereas Ro 20-5331, tested at WRAIR in the Aotus monkey, seems to be less active than mefloquine, our own studies carried out in Basle by Dr. Richle and complemented by Prof. Peters in Liverpool, make us believe that Ro 21-0960 and Ro 21-3473 are at least as active as *mefloquine*. Broader studies with both compounds are necessary before a decision regarding possible further development can be made.

Table 1 : Activity Against Normal Strains of Plasmodium berghei in Mice

		Ro 20-5331/002 (2HCl) Rac.	Ro 21-0960/000 Rac.	Ro 21-3473/000 Rac.	Mefloquine (HCl) Rac.	Quinine (HCl) nat.
DL 50 (mg/kg i.p.)		205	900	1000		210
Plasmodicidal dose on 3 days (MED, mg/kg p.o.) Courtesy of Prof. Brener, Belo Horizonte		50	12.5			200
Dosage on 4 days (mg/kg p.o.) Dr. Richle, Roche	ED 50	2.6	1.6		1.8	10[a]
	ED 90	7.0	2.6		4.0	100[a]
Dosage on 4 days (mg/kg) Courtesy of Prof. Peters	ED 50	3.2p.o. 3.3s.c.	2.3p.o. 2.4s.c.		1.7p.o. 2.0s.c.	25p.o. 24s.c.
	ED 90	7.8p.o. 6.8s.c.	3.7p.o. 4.5s.c.		3.1p.o. 3.7s.c.	55p.o.100s.c.
Rane test Courtesy of WRAIR Cured mice versus treated mice after the following single doses (mg/kg s.c.) [prolongation of survival time in days in brackets]	10		[4.5]	[9.5]	[12.5]	
	20	[2.9]	[8.2]	[10.8]	4/5[b] [9.4][e]	
	40	[7.3]	[10.3]	[12.5]	5/5[b] 1/5[e]	
	80	[9.3]	c) [10.9]	2/5	1/5[e]	
	160	0/5 [10.7]	[13.0]	3/5	4/5[e]	[2][d]
	320	3/5	[14.3]	5/5	4/5[e]	
	640	5/5	[16.2]	5/5	5/5[e]	[5.4][d]

a) Quinine sulfate b) J.Med.Chem. 14,926(1971) c) both optical isomers showed activities similar to Ro 21-3473

d) J.Med.Chem. 10,431(1967) e) J.Med.Chem. 17,210(1974)

Table 2 : Activity Against Chloroquine-Resistant Strains of Plasmodium berghei in Mice (Courtesy of Prof. Peters)

		Ro 20-5331/002 (2HCl) Rac.	Ro 21-0960/000 Rac.	Ro 21-3473/000 Rac.	Mefloquine (HCl) Rac.	Quinine (2HCl) nat.
NS-Strain Dosage on 4 days (mg/kg)	ED 50		4.0 p.o.		1.8 p.o.	55 p.o.
	ED 90	7.4p.o. 8.5s.c.	9.8 p.o.		6.0p.o. 10s.c.	200 p.o.
RC-Strain Dosage on 4 days (mg/kg)	ED 50		>30 p.o.		>30 p.o.	~ 600 s.c.
	ED 90	~450p.o.[a] >MTDs.c.[b]	>30 p.o.		>30 p.o. >30s.c.	>1000 s.c.

a) Graphically interpolated value

b) MTD = maximal tolerated dosage

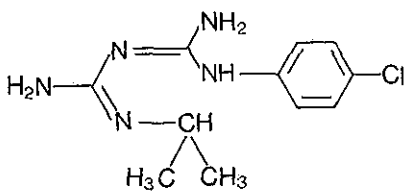
The biological activity of most natural products (e.g. the antiprotozoal agent emetine) is highly specific with regard to their stereochemistry. This seems not to be the case for Cinchona alkaloids. It may well be that the conversion of Cinchona alkaloids having a quinine configuration into compounds with a quinidine configuration takesplace by well-established metabolic pathways including oxidation of the C-9 hydroxy group followed by isomerization at C-8 and reduction of the carbonyl group formed. However, this hypothesis remains to be proven.

Whereas these approaches, using available knowledge and modern chemistry, may lead to improved drugs, it is questionable whether they will bring real breakthroughs, because all these compounds might interfere with nucleic acid synthesis as does quinine. The improved activity of synthetic quinine analogs may be concerned with interference in metabolic processes, this being achieved by alteration in the aromatic substituents. In this respect the trifluoromethyl group in position 2' probably plays an important role.

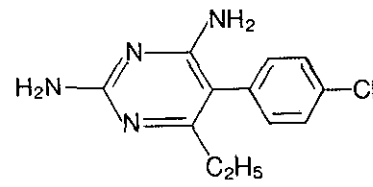
Basic Research :

The development of antifolics as antimalarials can be regarded as an example for this approach(7). Sulfonamides act as antimetabolites of p-aminobenzoic

acid in the condensation of a dihydropteridine derivative with p-aminobenzoic acid to form dihydropteroic acid. With glutamic acid, folic acid is formed and converted into dihydrofolic acid and tetrahydrofolic acid by an enzyme called folic acid reductase. Chlorguanide and pyrimethamine block the enzymatic reaction of folic acid reductase. The latter compounds have been introduced as antimalarial drugs after World War II with great success. They are, however, not useful in the treatment of acute malarial attacks.

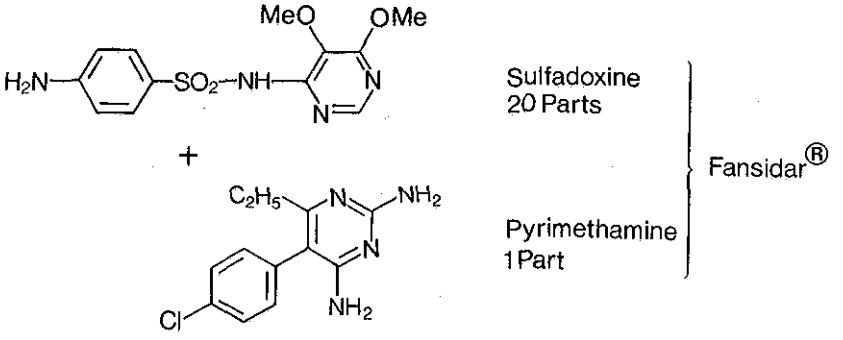


Chlorguanide



Pyrimethamine, Daraprim®

Combinations of sulfonamides with pyrimethamine or chlorguanide lead to potentiation of antimalarial effects, because they act at different points in the same metabolic pathway. The development of improved antifolates like Fansidar® (8) is both the result of basic research on the mode of action of drugs and of directed synthetic studies.



A combination therapy using compounds like quinine or some of its more potent derivatives together with antifolates does not seem unreasonable. Such treatment will undoubtedly interfere with the malaria parasites at completely different biological stages and should, therefore, result in an even slower development of malaria-resistant strains. Whether this will be the final answer, is not known. The malaria parasites may be smart enough to overcome this hurdle again, and the battle against malaria might only be won by constantly developing novel drugs or drug combinations interfering with the life-cycle of the parasite at always other stages.

It seems that those involved in developing new antimalarials can never rest. They have to be all the time active in the search for new chemical entities, which are better tolerated by the host and fatal for the parasite.

I would like to thank Drs. A. Rheiner, R. Richle, M. Fernex and U. Solms, F.Hoffmann-La Roche & Co., Ltd., Basle, Switzerland, and our collaborators from *Hoffmann-La* Roche Inc., Nutley, USA, especially Dr. M. Uskokovic, for helping me to present this report.

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