ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1994, p. 1169–1171
0066-4804/94/$04.00+0

Vol. 38, No. 5

In Vitro Activities of Furoquinoline and Acridone Alkaloids against *Plasmodium falciparum*

LEONARDO K. BASCO,[1]* SOFIA MITAKU,[2]† ALEXIOS-LEANDROS SKALTSOUNIS,[2]† NICOLE RAVELOMANANTSOA,[3] FRANÇOIS TILLEQUIN,[2] MICHEL KOCH,[2] AND JACQUES LE BRAS[1]

Laboratoire de Parasitologie, Institut de Médecine et d'Epidémiologie Africaines, Hôpital Bichat-Claude Bernard, 75877 Paris,[1] Laboratoire de Pharmacognosie, Unité de Recherche Associée au Centre National de la Recherche Scientifique no. 1310, Faculté de Pharmacie, Université de Paris V, 75270 Paris,[2] and Laboratoire Fibres Energie Biomonomères, Ecole Nationale Supérieure de Chimie de Toulouse, 31078 Toulouse,[3] France

Received 12 November 1993/Returned for modification 4 January 1994/Accepted 16 February 1994

The in vitro activities of furo[2,3b]quinoline and acridone alkaloids against *Plasmodium falciparum* were evaluated by an isotopic semimicrotest. A pyran ring in the furoquinoline nucleus and 2-*O*-pyranoglycoside and 2-nitro substituents in the acridone nucleus improved the antimalarial activities of the compounds. These findings provide a clue for further chemical modifications.

Malaria is one of the major parasitic infections in many tropical and subtropical regions. *Plasmodium falciparum* is becoming resistant to 4-aminoquinolines, antifols, and even aminoalcohols, and only a few alternative drugs are under development, necessitating urgent efforts to identify novel classes of antimalarial drugs (18). The clinical utility of quinine and quinidine isolated from *Cinchona* tree bark and the Chinese discovery of artemisinin from the herb *Artemisia annua* have stimulated much interest in plants as potential sources of new antimalarial drugs.

Furo[2,3b]quinoline and acridone alkaloids have been isolated from plants belonging to the *Rutaceae* family. Several synthetic furoquinolines have been shown to be active against rodent malaria (2), but to our knowledge, the furoquinoline alkaloids isolated from these plants have not been studied for their antimalarial activities. Acronycine exhibits antitumor activity (16), and acronycine derivatives have been tested for their activities against *Trichomonas vaginalis* (15), *Pneumocystis carinii* (13), and *Plasmodium yoelii* (4, 5). Several acridone alkaloids have been shown to be active against *P. yoelii* both in vivo and in vitro. The toxicity of acronycine has been studied in mice (3), and the clinical efficacy and tolerance of acronycine have been evaluated in a phase II study in cancer patients who received the drug daily for several months (14). To evaluate further the potential antimalarial activities of these alkaloids, we investigated the in vitro activities of 5 furoquinoline and 16 acridone alkaloids which have not been tested before for their antimalarial activities and compared their activities with those of acronycine and noracronycine.

The chloroquine-susceptible HB3 (Honduras) and the chloroquine-resistant W-2 (Indochina) clones of *P. falciparum* were kindly provided by D. Walliker of the Institute of Cell, Animal and Population Biology, University of Edinburgh. The clones were maintained in a continuous culture in human type O^+ erythrocytes in complete RPMI 1640 medium supplemented with 10% human serum and buffered with 25 mmol of HEPES

TABLE 1. In vitro activities of furoquinoline and acridone alkaloids against the chloroquine-susceptible HB3 and the chloroquine-resistant W2 clones of *P. falciparum*

Compound no. and the Alkaloids, and their derivatives[a]	IC_{50} (μg/ml)[b]	
	HB3	W-2
Furoquinoline		
1. Haplopine	8.90	8.34
2. Skimmiamine	15.7	14.1
3. Kokusaginine	23.1	7.70
4. Acronycidine	19.9	5.72
5. Acronydine	12.1	2.18
Acridone		
6. Normelicopidine	>100	>100
7. Melicopidine	14.1	5.54
8. Melicopicine	37.2	30.2
9. 1,3-Dihydroxy-*N*-methylacridone	>100	>100
Pyranoacridone		
10. Noracronycine	>100	>100
11. *N*-Desmethylacronycine	10.4	2.35
12. Acronycine	7.03	1.44
13. 2-Nitroacronycine	2.78	1.93
14. 1,2-Dihydroxy-1,2-dihydroacronycine	10.9	9.14
15. 1,2-Dihydroxy-1,2-dihydro-*N*-desmethyl-acronycine	7.92	6.05
16. 2-Hydroxy-1,2-dihydroacronycine	13.7	3.26
17. 2(−)1,2-Dihydroacronycine-2-yl(3-amino)-2,3,6-trideoxy-α-L-*arabino*, hexopyranose	4.86	0.600
18. 2(*R*)(−)1,2-Dihydroacronycine-2-yl(4-*O*-acetyl-3-bromo)-2,3,6-trideoxy-α-L-*arabino*, hexopyranose	3.45	1.78
19. 2(*S*)(−)1,2-Dihydroacronycine-2-yl(4-*O*-acetyl-3-bromo)-2,3,6-trideoxy-α-L-*arabino*, hexopyranose	6.94	1.46
20. 1-Methoxy-3-(2-methyl-2-propanaloxy)-9-acridanone-4-carbaldehyde	16.8	10.8
21. Dimer AB-2	>100	>100
22. Trimer AB-3	>100	>100
23. Dimer Diels-Alder adduct	>100	>100
Chloroquine	0.0103	0.149

[a] The numbers preceding the names of the derivatives correspond to the chemical structures presented in Fig. 1.

[b] Data are expressed as the mean IC_{50} from two separate determinations against the chloroquine-susceptible HB3 (Honduras) and the chloroquine-resistant W-2 (Indochina) clones.

* Corresponding author. Mailing address: Laboratoire de Parasitologie, Hôpital Bichat-Claude Bernard, 46 rue Henri Huchard, 75877 Paris Cedex 18, France. Fax: 33-1-46-27-02-08.

† Present address: Department of Pharmacy, Division of Pharmacognosy, University of Athens, Panepistimiopolis, Zografou, Athens 15771, Greece.

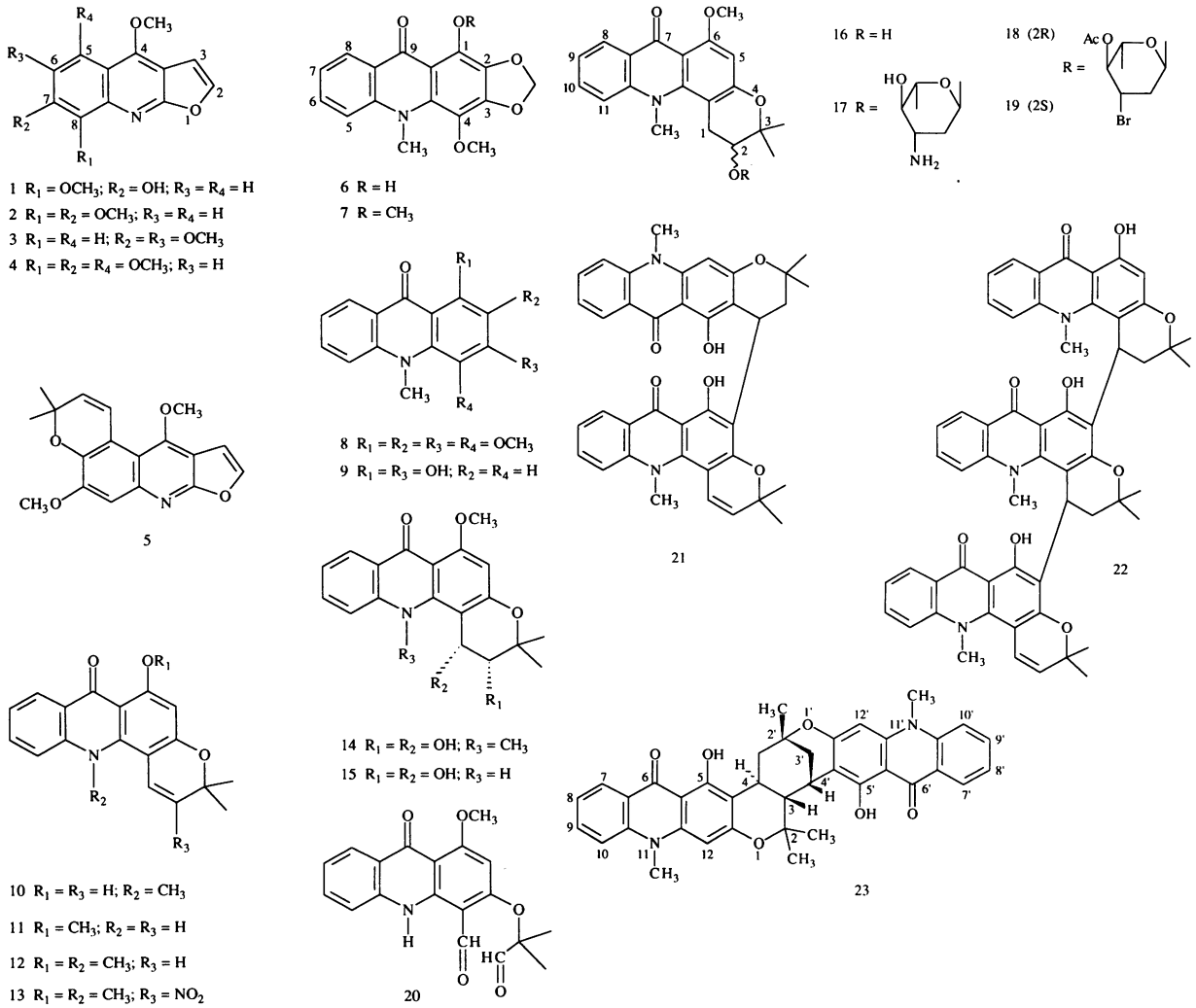

FIG. 1. Chemical structures of furoquinoline and acridone alkaloids.

(*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) per liter and 25 mmol of $NaHCO_3$ per liter (17).

Furoquinoline and acridone alkaloids were isolated from three New Caledonian plants, *Geijera balansae*, *Sarcomelicope glauca*, and *Sarcomelicope dogniensis* (9–12). The procedures of the chemical modifications and dimerization of acronycine were described elsewhere (1, 7, 8). The chemical structures of the alkaloids are presented in Fig. 1. Stock solutions of the alkaloids were prepared in methanol or dimethyl sulfoxide. Chloroquine sulfate (Specia, Paris, France) was dissolved in sterile distilled water. Twofold serial dilutions of the test compounds were prepared in sterile distilled water and were distributed in 24-well plates in triplicate. The in vitro drug susceptibility test of Le Bras and Deloron (6) was used in the study. The suspension of parasitized erythrocytes in complete RPMI 1640 medium (1.5% hematocrit, 0.3 to 0.5% parasitemia, 700 μl per well) was distributed in the plates, and the plates were incubated at 37°C in 5% O_2–5% CO_2–90% N_2 for 42 h. [^{3}H]hypoxanthine (1 μCi per well; Amersham, Buckinghamshire, United Kingdom) was used as an index for parasite growth. The 50% inhibitory concentration (IC_{50}) was defined as the drug concentration corresponding to 50% of the amount of [^{3}H]hypoxanthine incorporated by the parasites in the drug-free control wells. The activity of each test compound was determined twice against both clones. The results were expressed as the mean IC_{50} of two independent experiments.

The in vitro activities of 23 furoquinoline and acridone alkaloids are presented in Table 1. Fourteen alkaloids had IC_{50}s of <10 μg/ml against the chloroquine-resistant clone. The most active alkaloid, compound 17, an *O*-pyranoglycoside derivative of acronycine, had an IC_{50} of 0.60 μg/ml against the resistant clone. Ten compounds were more than twice as active against the resistant clone than the susceptible clone. Six compounds were inactive (IC_{50}s, >100 μg/ml). The activities of the furoquinoline alkaloids were within the range of activities of the acridone derivatives. These furoquinolines possess a methoxy group at the C-4 position. The functional groups at the C-5, C-6, C-7, or C-8 position of simple furoquinolines did not alter the antimalarial activity considerably. The presence of a pyran ring increased the activity against the resistant clone.

Acronycine was moderately active against *P. falciparum*. Among the 1,2-unsaturated tetracyclic acronycine derivatives, only the 2-nitro compound improved the activity of acronycine. The 1,2-saturated tetracyclic acronycine derivatives 14, 15, and 16 did not ameliorate the activity of acronycine. However, three 1,2-saturated 2-*O*-pyranoglycosides 17, 18, and 19 exhib-

ited activities similar to or greater than that of the parent compound. The monomeric, dimeric, and trimeric derivatives of noracronycine and tricyclic acridone alkaloids 6, 7, 8, 9, and 20 were either inactive or less active than the tetracyclic acronycine derivatives. The presence of a chelated hydroxy group in compounds 6, 9, 10, 21, 22, and 23 resulted in a complete loss of antimalarial activity. In contrast, the methoxy group in the same position was crucial for antimalarial activity. With the exception of *O*-pyranoglycoside and nitro substituents at C-2, the functional groups at the C-1, C-2, or N position in the acridone nucleus did not modify the antimalarial activity of the parent compound.

The results of the present study confirm the moderate in vitro activity of acronycine against *P. falciparum* and demonstrate the antimalarial activities of furoquinoline alkaloids. Although a highly active compound was not identified in this series, several leads were provided, notably, the importance of the 2-*O*-pyranoglycoside and 2-nitro substituents in the acridone nucleus and the pyran substituent in the furoquinoline nucleus. On the basis of these findings, further modifications of furoquinoline and acronycine that will ameliorate the antimalarial activities of these alkaloids are under study.

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