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Antimalarial Activities of Ring-Substituted Bioimidazoles†

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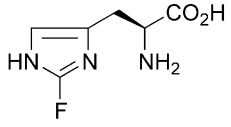
Abstract—We report in vitro antimalarial activities against chloroquine sensitive and resistant *Plasmodium falciparum* strains, and in vivo activities against *Plasmodium berghei* in mice for four series of ring-substituted-L-histidines and histamines.

Parasitic diseases have an overwhelming impact on public health in developing regions, and malaria has for a long time presented a very serious global health problem. Attempts to control mosquito vector, and the use of antimalarial drugs notwithstanding, there are still some 1.5–3 million fatalities, mostly children and women annually from malaria.[1] Out of the four species of Plasmodium that affect humans, *Plasmodium falciparum* is the most prevalent and pathogenic. Resistance of plasmodia to antimalarial drugs is now recognized as one of the major problems in the eradication of malaria. The rapid increasing resistance of *P. falciparum* malaria parasites to the most commonly used drug chloroquine has made it ineffective. Control of malaria is further hampered by emergence of resistance to new and more expensive chemotherapeutic agents, such as mefloquine and halofantrine, mosquitoes resistant to pesticides, and by restriction in the use of chemical sprays. Despite tremendous efforts an effective vaccine has not been found yet. The inadequate armory of drugs in widespread use for the treatment of malaria and lack of affordable new drugs are the limiting factors in the fight against malaria. This underscores the continuing need for new structural classes of antimalarial agents with novel and different mechanisms of action.

Asexual *P. falciparum* parasites are known to express within an invaded erythrocyte a number of unusual proteins including at least three histidine rich proteins (HRPs) containing high histidine content.[2] The HRP1 has been localized by immunoelectron microscopy to electron-dense knobs below the outer surface of the erythrocyte membrane.[3,4] The high histidine content create centers of very high positive charge; thus knobs may be responsible for a very strong adherence of the infected erythrocytes to the capillary endothelium, thereby sequestering parasitized cells, which would normally be destroyed during passage through the spleen.[5] Thus, HRP1 appears to play an important structural and/or functional role in knobs and is possibly advantageous for parasite survival.

2-Fluoro-L-histidine (2-FHIS, **1**, Fig. 1) was the first compound chosen for antimalarial testing because, Torrence et al.[6] had previously demonstrated that 2-FHIS (**1**) can partially replace histidine in de novo protein in both *Escherichia coli* and mammals. Furthermore, the fluorine atom reduces the p*K* of the imidazole ring from its normal value of 6 to 1.5;[7] thus, its incorporation into knobs should dilute the clusters of positive



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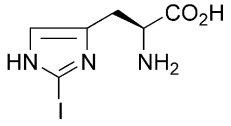

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Figure 1.

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charge and reduce cytoadherence. In cultures of infected erythrocytes, low concentrations of 2-FHIS (**1**) not only inhibit cytoadherence but also prevent maturation of the parasites and the appearance of knobs entirely.[8] The assumption that these strong antiparasitic effects are due to incorporation of 2-FHIS proved unwarranted, since the treated parasite shows a rather low incorporation of labeled 2-FHIS, but a general decrease in protein synthesis. As one of the several mechanism of action, it was proposed that 2-FHIS interferes with histidine on the outer cell surface as a promoter of the other essential amino acids (especially isoleucine) into the cell.[6] Unfortunately, the high antimalarial activity shown by 2-FHIS in vitro could not be extended because the compound proved lethal to owl monkeys, even at dose 1/20 that of the LD_{50} for mice (250 mg/kg).[6]

The excellent in vitro antimalarial activity shown by 2-FHIS prompted us to explore other halogenated analogues of histidine and its decarboxylated product histamine for their antimalarial activity. Biological testing of all synthesized ring-halogenated compounds[9] indicated that only 2-iodo-L-histidine analogue (2-IHIS, **2**, Fig. 1) showed activity comparable to that of 2-FHIS (**1**). Neither 5-halohistidines nor 2,5-dihalohistidines showed activity, while 2-iodohistamine was somewhat active; surprisingly, the 2-chloro and 2-bromo analogues were inactive.[10] Thus, the role of iodine atom cannot be electronic (Cl, Br and I have about the same electronegativity and the same effect on imidazole ring p*K*).

Parasite matures and multiplies within the red blood cells and feeds on hemoglobin and other component of the erythrocyte. However, it must import certain nutrients from outside the cell and has developed one or more ducts in the erythrocyte membrane, which it uses as 'mouth.'[11] It is proposed that 2-IHIS (**2**) may just be the right size to plug an erythrocyte membrane channel involved in active or passive transport of nutrients essential for parasite survival. In contrast to 2-FHIS (**1**), 2-IHIS (**2**) does not significantly retard protein synthesis while retarding parasite maturation, a result supporting this mechanism of action. Although, 2-IHIS (**2**) proved non-toxic to monkeys, it retarded growth of the parasite for only 24 h. It was later demonstrated that iodine is rapidly removed non-enzymatically under physiological conditions by any sulfhydryl compound (cysteine, glutathione, etc.) present in the tissue.[12] The finding that deiodination need not be enzyme-mediated greatly reduces the possibility of stabilizing the molecule by derivatization of the side chain, by use of the D-histidine series, or by introduction of a bulky alkyl group.

The malaria parasite has developed resistance to drugs by creating an energy driven pump, which expels drugs as soon as it penetrates the erythrocyte. Whether, the drug enters and is expelled through the 'ducts' or through another conduit, is yet uncertain.[11] Examination of space filling models revealed that 2-iodo-L-histidine (**2**) has a width corresponding exactly to the diameter of the erythrocyte membrane channel, as

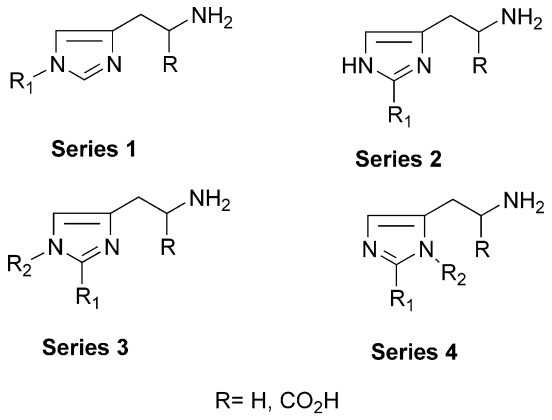

Figure 2.

estimated from diffusion rates of small molecules.[13,14] The same dimension can be found in metabolically stable molecules, such as 1-isopropyl and 2-isopropylhistidine. Thus, replacement of metabolically unstable iodine at the imidazole ring with more stable functions of similar size and lipophilicity may lead to antimalarial compounds which block parasite maturation in an infected erythrocyte by plugging a nutrient channel or duct. This approach may bypass the ability of the parasite to eject a drug and may even permit the use of combination therapy, by preventing certain previously effective antimalarial agents from being ejected.

On the assumption that iodine could be replaced by sterically equivalent, but metabolically stable alkyl group, we initiated a program to develop general synthetic methods for the previously inaccessible ring-alkylated histidines and histamines (Series 1–4, Fig. 2), and this paper describes the antimalarial activities of the synthesized analogues.

All four series were synthesized according to procedures developed earlier in our laboratory.[15–18] Thus, 1-alkylbioimidazoles [Series 1, **3**–**12**, $R_1 = CH_3$, C_2H_5, $CH_2CH{=}CH_2$, $CH(CH_3)_2$, $CH_2C_6H_5$] were synthesized by the alkylation of the corresponding 5,6,7,8-tetrahydro-5-oxoimidazo[1,5-*c*]pyrimidines followed by the acidolysis of the respective quaternary salt as described in the procedure reported earlier.[15] Whereas, 2-alkylbioimidazole analogues [Series 2, **13**–**27**, $R_1 = CH_3$, C_2H_5, $CH(CH_3)_2$, $C(CH_3)_3$, cycloalkyl group, adamantyl] were obtained by regiospecific C-2 alkylation of the fully protected L-histidine and histamine via silver catalyzed radical decarboxylative oxidation of alkylcarboxylic acids by ammonium persulfate in 10% sulfuric acid.[16]

Extension of the methodologies[15,16] resulted in the synthesis of 1,2-dialkylbioimidazole analogues (Series 3, **28**–**37**, R_1 = alkyl, cycloalkyl group, $R_2 = CH_3$, $CH_2C_6H_5$) as reported earlier.[17] Similarly, phenacyl-directed regiospecific N-3 (π) alkylation led to the synthesis of 2,3-dialkyl-L-histidine and histamine analogues (Series 4, **38**–**47**, R_1 = alkyl, cycloalkyl, $R_2 = CH_3$, $CH_2C_6H_5$).[18]

Table 1. In vitro sensitivity of chloroquine sensitive *P. falciparum* strain to ring-substituted bioimidazoles

No.	R	R_1	R_2	IC_{50} (μM)
Series 1				
3	CO_2H	CH_3	—	> 500
4	CO_2H	C_2H_5	—	> 500
5	CO_2H	$CH_2CH{=}CH_2$	—	nd
6	CO_2H	$CH(CH_3)_2$	—	120
7	CO_2H	$CH_2C_6H_5$	—	360
8	H	CH_3	—	> 500
9	H	C_2H_5	—	> 500
10	H	$CH_2CH{=}CH_2$	—	176
11	H	$CH(CH_3)_2$	—	9.6
12	H	$CH_2C_6H_5$	—	20.7
Series 2				
13	CO_2H	CH_3	—	> 500
14	CO_2H	C_2H_5	—	> 500
15	CO_2H	$CH(CH_3)_2$	—	6.3
16	CO_2H	$C(CH_3)_3$	—	25.8
17	CO_2H	*c*-C_4H_7	—	255
18	CO_2H	*c*-C_5H_9	—	276
19	CO_2H	*c*-C_6H_{11}	—	350
20	CO_2H	Adamantyl	—	450
21	H	CH_3	—	> 500
22	H	C_2H_5	—	> 500
23	H	$CH(CH_3)_2$	—	4.7
24	H	$C(CH_3)_3$	—	15.5
25	H	*c*-C_4H_7	—	367
26	H	*c*-C_5H_9	—	489
27	H	*c*-C_6H_{11}	—	> 500
Series 3				
28	CO_2H	$CH(CH_3)_2$	CH_3	> 500
29	CO_2H	$C(CH_3)_3$	CH_3	> 500
30	CO_2H	*c*-C_6H_{11}	CH_3	> 500
31	CO_2H	$CH(CH_3)_2$	$CH_2C_6H_5$	89
32	CO_2H	$C(CH_3)_3$	$CH_2C_6H_5$	157
33	CO_2H	*c*-C_6H_{11}	$CH_2C_6H_5$	nd
34	H	$CH(CH_3)_2$	CH_3	387
35	H	$C(CH_3)_3$	CH_3	459
36	H	*c*-C_6H_{11}	CH_3	> 500
37	H	*c*-C_3H_5	CH_3	nd
Series 4				
38	CO_2H	$C(CH_3)_3$	CH_3	> 500
39	CO_2H	*c*-C_6H_{11}	CH_3	nd
40	CO_2H	$CH(CH_3)_2$	$CH_2C_6H_5$	355
41	CO_2H	$C(CH_3)_3$	$CH_2C_6H_5$	399
42	CO_2H	*c*-C_6H_{11}	$CH_2C_6H_5$	> 500
43	CO_2H	Adamantyl	$CH_2C_6H_5$	> 500
44	H	$CH(CH_3)_2$	$CH_2C_6H_5$	188
45	H	$C(CH_3)_3$	$CH_2C_6H_5$	233
46	H	*c*-C_6H_{11}	$CH_2C_6H_5$	> 500
47	H	Adamantyl	$CH_2C_6H_5$	> 500
Chloroquine				12 nM

nd, not determined.

In vitro antimalarial activities of bioimidazole compounds (Series 1–4) as IC_{50} values for the inhibition of chloroquine sensitive and resistant *P. falciparum* strains are summarized in Tables 1 and 2.[19]

As expected, 2-isopropyl-L-histidine (**15**, Series 2), the isosteric replacement of 2-iodo-L-histidine (**2**) exhibited antimalarial activity at 6.3 and 6.5 μM against chloroquine sensitive and resistant *P. falciparum* strains. However, the most effective compound of this series was 2-isopropylhistamine (**23**, Series 2) with IC_{50} values of 4.7 and 4.8 μM, respectively, for sensitive and resistant *P. falciparum* strains. To our surprise, 1-isoprpoyl-

Table 2. In vitro antimalarial activity against chloroquine resistant *P. falciparum* strain

No.	R	R_1	R_2	IC_{50} (μM)
11	H	$CH(CH_3)_2$	—	10.4
12	H	$CH_2C_6H_5$	—	22.5
15	CO_2H	$CH(CH_3)_2$	—	6.5
16	CO_2H	$C(CH_3)_3$	—	38.5
23	H	$CH(CH_3)_2$	—	4.8
24	H	$C(CH_3)_3$	—	30.3
31	CO_2H	$CH(CH_3)_2$	$CH_2C_6H_5$	245.3

Table 3. In vivo antimalarial activity against *P. berghei* in mice

No.	R	R_1	R_2	Comment
11	H	$CH(CH_3)_2$	—	Active at 100 mg/kg
12	H	$CH_2C_6H_5$	—	Inactive
15	CO_2H	$CH(CH_3)_2$	—	Active at 100 mg/kg
16	CO_2H	$C(CH_3)_3$	—	Inactive
23	H	$CH(CH_3)_2$	—	Active at 100 mg/kg
24	H	$C(CH_3)_3$	—	Inactive
31	CO_2H	$CH(CH_3)_2$	$CH_2C_6H_5$	Inactive

histamine (**11**, Series 1) exhibited significant antimalarial activity with IC_{50} values of 9.6 and 10.4 μM, whereas, 1-isopropyl-L-histidine (**6**, Series 1) was found to be less active with IC_{50} of 120 μM. The observation of quite significant activity in the histamine derivatives suggests that the carboxy group of the amino acid may be unnecessary for antimalarial activity. Likewise, potent antimalarial activities of histamine derivatives suggest that simple imidazoles with appropriate substitution at C-2 position may also show antimalarial activity. Finally, placement of more than one alkyl group in the imidazole ring (Series 3 and 4) resulted in compounds with drastically reduced antimalarial activity. Based upon in vitro antimalarial activity, compounds with IC_{50} values of less than 100 μM were selected for in vivo antimalarial activity evaluation against *Plasmodium berghei* infection in mice model (Table 3).[20]

Selected compounds were evaluated at a dose of 100 mg/kg/day × 4 and a maximum of six mice were used. Chloroquine (8 mg/kg/day × 4) was kept as standard drug in trial for comparison. Along the expected lines, 1-isopropylhistamine (**11**), 2-isopropyl-L-histidine (**15**) and 2-isopropylhistamine (**23**) were found to be active with all mice surviving with negative parasitemia up to day seven. However, none of the compounds was found to possess curative activity as all mice died before D + 28.

The results obtained established that some of the bioimidazole compounds have shown significant in vitro and in vivo antimalarial activity, confirming the hypothesis that iodine at C-2 position of imidazole ring can be successfully replaced by a metabolically stable group of comparable size. Furthermore, the results also indicate that to retain antimalarial activity, it is not necessary for a metabolically stable group to be present at C-2 position of the imidazole ring, but can be placed at N-1(τ). On the other hand, the properties of diffusion

ducts in the membrane of the infected erythrocyte suggests that effective antimalarials should carry both positive charge and the lipophilicity.[11] Since effective analogues already carry positive charge, efforts towards attachment of long fatty acid chains to the most effective compounds are underway. It can be concluded that this class of compounds certainly holds great prospects, and that further exploration in this field may lead to potent antimalarial agents.

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19. Different drug dilutions [test compounds and chloroquine (positive control)] were prepared in complete RPMI (medium RPMI 1640 + 10% AB^+ human serum; CRPMI). Fifty μL of each dilution was transferred to the respective well of a microtiter plate in triplicates. Parasitized erythrocytes (PE; mainly rings; 4% parasitaemia; 5% hematokrit) were added to each well. Volume in each well was made up to 200 μL with CRPMI. The plates were incubated at 37 °C in a candle jar. After 24–48 h of incubation, thin smears from each well were made and stained with Giemsa. The number of PE/10,000 cells was counted. Percent inhibition by the drug over the control (well which does not contain any drug) was plotted against the respective logarithmic concentration of the drug. Using non-linear regression analysis, the IC_{50} of the test compounds was then calculated.
20. On day '0', groups of six mice each were inoculated intraperitoneally with 1×10^7 infected-erythrocytes from a donor mouse. Four hours later, mice were administered test compounds/chloroquine/vehicle, orally. A total of four doses were given orally on days D '0', D + 1, D + 2, and D + 3. The tail blood smears were made on day D + 4 and D + 7, stained with Giemsa and examined microscopically. The minimum dose that completely suppressed parasitaemia on days D + 4 and D + 7 was termed as minimum effective dose (MED), and the minimum dose that cleared the parasitaemia for up to 28 days was termed as curative dose (CD). The terms 'curative', 'active' and 'inactive' are used to describe the biological activities exhibited by the tested compounds. The term 'curative' indicates complete elimination of malaria parasites from the body, so that relapse cannot occur up to day D + 28. The term 'active' indicates that the treated animals show negative parasitaemia up to D + 7. However, by D + 28, some mice show negative and some mice may show positive test result for parasitaemia. The term 'inactive' indicates that the treated animals show positive test result for parasitaemia either on D + 4 or D + 7 or on both D + 4 and D + 7.