Flavins as Potential Antimalarials. 1. 10-(Halophenyl)-3-methylflavins

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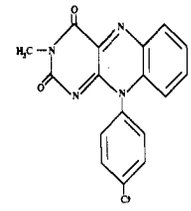
A series of 10-(halophenyl)-3-methylflavins was prepared by the condensation of 6-(haloanilino)-3-methyluracils with nitrosobenzene. A number of these flavins effectively cured lethal *Plasmodium vinckei* malarial infections in mice when administered by either oral or intraperitoneal routes.

We have recently examined a number of potential riboflavin antagonists for antimalarial activity.[1] Several 5-deazaflavins, including 5-deazariboflavin, were found to be inactive in vivo, while 10-(4-chlorophenyl)-3-methylflavin (1) was very active against *Plasmodium vinckei* in mice by both oral and intraperitoneal (ip) administration and against *Plasmodium falciparum* in culture. Our rationale for testing such compounds was based on the following: (1) Seeler and Ott's finding that *Plasmodium lophurae* growth was suppressed in riboflavin deficient chicks;[2] (2) Thurnham and co-workers' evidence that riboflavin deficiency could suppress *P. falciparum* infections in humans[3] and *Plasmodium berghei* infections in rats[4] and (3) Jensen and others' findings that 5-deazariboflavin inhibits the growth of *P. falciparum* in culture.[5]

In the present work we report the preparation of a number of 10-(halophenyl)flavins and their antimalarial activity in mice against *P. vinckei.*



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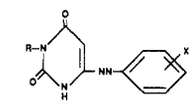

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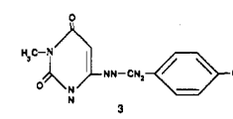

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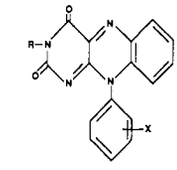

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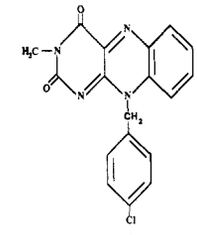

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Chemistry

The flavins were prepared by the action of nitrosobenzene on 6-anilinouracils in the presence of acetic anhydride, essentially according to the method of Yoneda et al.[6] The 6-anilino-3-methyluracils (**2a–k**; Table I), prepared by heating the appropriate aniline with 6-chloro-3-methyluracil, were isolated from ethanol and recrystallized from acetic acid. 6-(4-Chlorobenzyl)-3-methyluracil (**3**) was similarly prepared from 4-chlorobenzylamine and 6-chloro-3-methyluracil. Transamination of 6-aminouracil with 4-chloroaniline gave 6-(4-chloroanilino)uracil (**2l**; Table I). With the exception of the last named compound, yields in these reactions were good.

The syntheses of some of the flavins were complicated by poor solubilities of the anilinouracil substrates, especially the 2,4-dichloro derivative. This problem was overcome by increasing the volume of acetic acid in the reaction mixture; otherwise, yields were low and considerable difficulty was encountered in separating product from starting material. Yields of the flavins were generally acceptable (Table II).

Results and Discussion

Initial mouse screening tests revealed two very active substances and gave insight into structure–activity relationships for this series of novel antimalarials. Tables III and IV compare biological data for the current series of compounds with 10-(4-chlorophenyl)-3-methylflavin (1), which we had previously found to be very active against *P. falciparum* in vitro and *P. vinckei* in vivo.[1]

Most structural modifications were made to the 10-phenyl substituent, and in light of the considerable activity found previously[1] for compound 1, we first prepared and tested the 2′- and 3′-chlorophenyl isomers (**4a** and **4b**; Table II). The latter compound showed weak activity when administered orally (Table III) but was inactive when given intraperitoneally (Table IV), while the 2′-chloro analogue was ineffective over the same dose ranges by either route. Unlike the 3′-chloro compound, the 3′-bromo analogue (**4c**; Table II) was moderately active by both routes of administration, while the 4′-bromo isomer (**4d**; Table II) proved to be highly active by either route (Tables III and IV). The 3′-fluoro analogue (**4e**; Table II) was moderately active by the oral route but showed only weak activity when given ip. The 4′-fluoro isomer (**4f**; Table II) was rather ineffective by either route.

The order of activity of the 4′-halo derivatives, **4d > 1 > 4f**, shows increasing potency with increasing size of the 4′-substituent. The relationship of the 3′-halo derivatives is not as clear since the order of activity orally is **4c = 4e > 4b** and intraperitoneally is **4c > 4e ≥ 4b**. The only 2′-derivative tested (**4a**; Table II) was devoid of activity.

The dichloro derivatives showed a wide range of activity. The 3′,5′-dichloro compound **4j** proved to be the most effective member of this series and the most active flavin examined thus far. This agent cured all of the treated animals in each test dose when given intraperitoneally (Table IV) and was only slightly less effective in the oral tests (Table III). The 2′,5′-dichloro isomer **4i**, in contrast,

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Table I. Physical Properties of 6-(Haloanilino)uracils **2**

no.	X	R	mp, °C	% yield	formula	anal.
2a	4-Cl	CH_3	340–342[a]	68	$C_{11}H_{10}ClN_3O_2$	C, H, N
2b	2-Cl	CH_3	323–324	88	$C_{11}H_{10}ClN_3O_2$	C, H, N
2c	3-Cl	CH_3	295–297	87	$C_{11}H_{10}ClN_3O_2$	C, H, N
2d	3-Br	CH_3	291–292	74	$C_{11}H_{10}BrN_3O_2$	C, H, N
2e	4-Br	CH_3	335–336	38	$C_{11}H_{10}BrN_3O_2$	C, H, N
2f	3-F	CH_3	330–331	68	$C_{11}H_{10}FN_3O_2$	C, H, N
2g	4-F	CH_3	343–344	79	$C_{11}H_{10}FN_3O_2$	C, H, N
2h	2-CH_3, 4-Cl	CH_3	306–307	87	$C_{12}H_{12}ClN_3O_2$	C, H, N
2i	2,4-Cl_2	CH_3	370–372	77	$C_{11}H_9Cl_2N_3O_2$	C, H, N
2j	2,5-Cl_2	CH_3	314–316	81	$C_{11}H_9Cl_2N_3O_2$	C, H, N
2k	3,5-Cl_2	CH_3	328–330	74	$C_{11}H_9Cl_2N_3O_2$	C, H, N
2l	4-Cl	H	338–339	32	$C_{10}H_8ClN_3O_2$	C, H, N

[a] Literature[6] mp 297 °C.

Table II. Physical Properties of 10-(Halophenyl)flavins **4**

no.	X	R	mp, °C	% yield	formula	anal.
4a	2-Cl	CH_3	367–368	53	$C_{17}H_{11}ClN_4O_2$	C, H, N
4b	3-Cl	CH_3	365–366	41	$C_{17}H_{11}ClN_4O_2$	C, H, N
4c	3-Br	CH_3	351–353	47	$C_{17}H_{11}BrN_4O_2$	C, H, N
4d	4-Br	CH_3	>370	40	$C_{17}H_{11}BrN_4O_2$	C, H, N
4e	3-F	CH_3	>370	47	$C_{17}H_{11}FN_4O_2$	C, H, N
4f	4-F	CH_3	>370	59	$C_{17}H_{11}FN_4O_2$	C, H, N
4g	2-CH_3, 4-Cl	CH_3	317–320	20	$C_{18}H_{13}ClN_4O_2$	C, H, N
4h	2,4-Cl_2	CH_3	347–348	40	$C_{17}H_{10}Cl_2N_4O_2$	C, H, N
4i	2,5-Cl_2	CH_3	357–359	43	$C_{17}H_{10}Cl_2N_4O_2$	C, H, N
4j	3,5-Cl_2	CH_3	>370	40	$C_{17}H_{10}Cl_2N_4O_2$	C, H, N
4k	4-Cl	H	>370	29	$C_{16}H_9ClN_4O_2$	C, H, N
4l[a]	3,4-Cl_2	CH_3	>370	48	$C_{17}H_{10}Cl_2N_4O_2$	C, H, N

[a] Literature[6] mp >360 °C.

Table III. Oral Antimalarial Activity of 10-(Halophenyl)-3-methylflavins against *P. vinckei vinckei* in Mice[a]

	percent cured and increase in mean survival (days) at dose, mg/kg				
drug[b]	25	50	75	100	150
1[c]	45 (2.4)	75 (1.4)	85 (1.0)	90 (2.7)	90 (2.7)
4a	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4b		0 (0)	20 (4.3)	0 (1.2)	60 (2.5)
4c	0 (1.0)	20 (2.0)	40 (1.5)	60 (1.5)	60 (1.0)
4d	60 (3.5)	100	100	100	100
4e	40 (0)	40 (0)	20 (2.5)	80 (0)	40 (0)
4f	0 (0)	0 (0.8)	0 (0.6)	0 (1.0)	20 (2.3)
4g	0 (0.5)	80 (1.5)	40 (3.2)	60 (2.0)	40 (1.5)[d]
4h	0 (0)	0 (1.3)	40 (1.0)	80 (1.5)	60 (1.5)
4i	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4j	60 (3.5)	100	100	100	100
4k	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4l	0 (0)	0 (0)	0 (0.5)	0 (0.5)	40 (1.2)

[a] Animals were considered cured if still living 60 days posttreatment with a single oral dose in propylene glycol (50 μL). [b] For comparison chloroquine diphosphate cured all treated animals at doses of 25 mg/kg or higher. [c] This compound included for comparison (results out of 20 mice); see ref 1. [d] Toxic.

Table IV. Parenteral Antimalarial Activity of 10-(Halophenyl)-3-methylflavins against *P. vinckei vinckei* in Mice[a]

	percent cured and increase in mean survival (days) at dose, mg/kg				
drug[b]	10	15	20	25	30
1[c]	60 (1.5)	95 (1.0)	100	90 (2.0)	
4a	0 (0)	0 (0)	0 (−0.2)[d]	0 (−1.4)[d]	
4b		0 (0)	0 (0.2)	0 (0)	0 (0)
4c		0 (1.3)	20 (1.4)	60 (3.0)	60 (1.0)
4d	80 (2.0)	100	100	60 (14.0)	40 (13.0)
4e	0 (0)	0 (0.2)	40 (1.7)	0 (2.0)	0 (2.2)
4f	0 (0)	0 (0.8)	20 (0.5)	0 (0.8)	0 (0.2)
4g		0 (1.2)	0 (1.2)	0 (−1.4)[d]	0 (−2.0)[d]
4h	0 (1.7)	0 (0.5)	0 (0)	0 (−1.2)[d]	0 (−1.0)[d]
4i	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4j[e]	100	100	100	100	100
4k	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4l	0 (0.5)	60 (0.5)	100	80 (0)	60 (1.0)[d]

[a] Animals were considered cured if still living 60 days posttreatment with a single intraperitoneal injection in olive oil (100 μL). [b] At 10 mg/kg chloroquine diphosphate gave 80 (2.0) and cured all animals treated at doses of 15 mg/kg or higher. [c] This compound included for comparison (results out of 25 mice); see ref 1. [d] Toxic. [e] Results out of 10 treated mice.

was completely ineffective at all test doses by either route of administration. The order of activity for the 2′,4′- and 3′,4′-dichloro isomers depended upon their route of administration. Thus the 2′,4′-dichloro derivative **4h** had moderate activity when given orally (Table III) but was virtually inactive or toxic by the ip route. The reverse held for the 3′,4′ isomer **4l**, which had considerable activity by the intraperitoneal route but was not very effective when given orally.

A noteworthy observation made for the disubstituted compounds is the deleterious effect that 2′-substituents have on activity. For example, introduction of a 2′-methyl (**4g**) or 2′-chloro group (**4h**) into the parent 4′-chloro compound **1** resulted in a considerable loss in activity (Table IV). In keeping with this observation, compounds **4a** and **4i**, which have 2′-chloro groups, were inactive.

The *N*-methyl substituent in the reference compound **1** was necessary for antimalarial activity since the desmethyl 4′-chlorophenyl derivative **4k** was completely ineffective by either route of administration at all test doses. Furthermore, direct attachment of the halophenyl group to N-10 was also necessary for activity since compound **5**, which has a methylene linker between the flavin moiety and the 4-chlorophenyl group, was devoid of antimalarial activity over the entire test dose range by either route of administration.

Conclusions

The small number of compounds examined in this study places obvious limits on delineating structure–activity relationships. However, some trends for this series are

apparent, for example: (1) for compounds with 4′-halo substituents the order of antimalarial activity is Br > Cl > F; (2) 2′-substituents lower or abolish activity. Furthermore, of the 4′-chlorophenyl substituted compounds it is clear that (1) the 3-methyl group is required for activity and (2) the halophenyl group must be directly attached to N-10, and, additionally, we previously showed that N-5 was required for activity since the 5-deaza analogue of **1** was inactive.[1]

The most active compounds in this series, **4d** and **4j**, will be further tested against other plasmodial species and against chloroquine-resistant strains of *P. falciparum* and *P. vinckei*. Initial results of trials against the latter strain appear encouraging.[7]

Experimental Section

Melting points are uncorrected. Analyses were performed by the Analytical Service Unit in the Australian National University and C, H, N values were within ±0.4% of the theoretical. Nitrosobenzene, 6-aminouracil, and the anilines used herein were obtained from Aldrich Chemical Co. or Aldrich-Chemie.

Antimalarial Screening in Mice. The 10-(halophenyl)-3-methylflavins were tested against a normal drug-sensitive strain of *P. vinckei vinckei* (strain V52, from F.E.G. Cox, Kings College, London), which had been passaged several times before use. Female CBA/CaH mice, 6–8 weeks old, were used in all in vivo experiments. Infections were initiated by ip injection of 5×10^5 parasitized red blood cells and monitored by examining Giemsa-stained thin smears. Infections became patent on the fourth day and rose exponentially to reach 40–50% parasitemia by day 7, death always occurring within a further 2–3 days.

To test for antimalarial activity after oral administration, the finely powdered drugs were suspended in propylene glycol and administered to groups of five mice by gavage (50 μL). A single dose was given when parasitemias reached 30–40%, and blood smears were examined 24 and 48 h later. Control animals received propylene glycol alone. Animals were considered cured when no parasites were evident 60 days after treatment. These results are summarized in Table III. Similarly, to test for antimalarial activity after parenteral administration, the powdered drugs were suspended in olive oil and administered as a single dose by ip injection (100 μL). Control animals received olive oil alone. These results are summarized in Table IV.

6-Anilino-3-methyluracils 2a–k. An intimate mixture of 6-chloro-3-methyluracil[8] (1.6 g, 10 mmol) and the appropriate aniline (30 mmol) was heated in an oil bath at 170–175 °C for 15 min (155–160 °C for 10 min for compound **2e**), cooled briefly, and poured into ethanol (35 mL) and stirred for 15 min. The solid was filtered off, washed with ether (2 × 30 mL), recrystallized from acetic acid, and dried to give **2a–k** (Table I). Analytical samples were recrystallized from MeOH.

6-(4-Chloroanilino)uracil (2l). A mixture of 6-aminouracil (5.1 g, 40 mmol), 4-chloroaniline (10.9 g, 85.4 mmol), and acetic acid (2 mL) was heated at an internal temperature of 205–210 °C for 3 h. The melt was cooled and poured into ethanol (35 mL) and stirred for 15 min. The solid was filtered off, washed with ethanol, and recrystallized from acetic acid to give **2l** (3.0 g, 32%): mp 317–318 °C (from AcOH/MeOH); IR (KBr) 3425, 3210, 1765, 1725, 1625, 1405, 1300, 1240 cm^{-1}; UV λ_{max} (log ε) (EtOH) 224 (4.00), 264 (4.37); MS (EI), *m/e* (relative intensity) 237, 239 (M^+, 100, 33), 152 (52), 127 (84), 68 (70). Anal. ($C_{10}H_8ClN_3O_2$) C, H, N.

6-[(4-Chlorobenzyl)amino]-3-methyluracil (3). 6-Chloro-3-methyluracil (2.0 g, 12.5 mmol) was refluxed with 4-chlorobenzylamine (4.43 g, 31.3 mmol) in 1-butanol (50 mL) for 4 h. After cooling to room temperature, the mixture was poured into petroleum ether (bp 40–60 °C, 100 mL) and stirred for 10 min. The solid was filtered off, washed with water, dried, and recrystallized from EtOH to give **3** (2.0 g, 60%): mp 317–319 °C (from MeOH); IR (KBr) 3460, 3380, 1745, 1700, 1655, 1580, 1470, 1325, 755, 655 cm^{-1}; UV λ_{max} (log ε) (EtOH) 224 (4.26), 266 (4.39); MS (EI), *m/e* (relative intensity) 265, 267 (M^+, 15, 5), 127 (33), 125 (100). Anal. ($C_{12}H_{12}ClN_3O_2$) C, H, N.

10-(Halophenyl)flavins 4a–g, 4i, and 4j. The appropriate 6-anilino-3-methyluracil (**2b–k**) (10 mmol) and nitrosobenzene (3.21 g, 30 mmol) were refluxed in a mixture of acetic anhydride (16 mL) and acetic acid (6 mL) for 35 min. The volume of the reaction mixture was then reduced by ca. 50% under reduced pressure and ethanol (15 mL) added. After crystallization was complete, the solid was filtered off, washed with ethanol and ether, and recrystallized from acetic acid to give compounds **4a–g**, 4i, and **4j** (Table II). Analytical samples were prepared from MeOH.

10-(2,4-Dichlorophenyl)-3-methylflavin (4h). Nitrosobenzene (1.6 g, 15 mmol) and **2i** (1.42 g, 5 mmol) were refluxed in a mixture of acetic anhydride (10 mL) and acetic acid (25 mL) for 1.5 h. The volume was reduced to ca. 10 mL under reduced pressure and the mixture treated as above to give **4h** (Table II). The analytical sample was recrystallized from acetic acid: IR (KBr) 3470, 3090, 1720, 1670, 1625, 1590, 1570, 1490, 1280, 760 cm^{-1}; UV λ_{max} (log ε) (EtOH) 268 (4.38), 339 (3.86), 437 (3.95); MS (EI), *m/e* (relative intensity) 374 (M^+, 4), 337 (100), 280 (57), 252 (52).

10-(4-Chlorobenzyl)-3-methylflavin (5). Nitrosobenzene (1.6 g, 15 mmol) and **3** (1.33 g, 5 mmol) were refluxed for 1 h in a mixture of acetic anhydride (8 mL) and acetic acid (2 mL). The mixture was worked up as for the 10-(halophenyl)flavins above to give, after two recrystallizations from acetic acid, **5** (0.85 g, 53%): mp 280–282 °C (from MeOH); IR (KBr) 3470, 1715, 1665, 1560, 1285, 760 cm^{-1}; UV λ_{max} (log ε) (EtOH) 268 (4.35), 314 (4.05), 436 (3.74); MS (EI), *m/e* (relative intensity) 352, 354 (M^+, 100, 34), 228 (49), 89 (34). Anal. ($C_{18}H_{13}ClN_4O_2$) C, H, N.

10-(4-Chlorophenyl)flavin (4k). Treatment of **2l** and nitrosobenzene as for compound **5** above gave **4k** (Table II): IR (KBr) 3470, 1715, 1650, 1555, 1420, 1285, 840, 770 cm^{-1}; UV λ_{max} (log ε) (EtOH) 213 (4.42), 267 (4.41), 338 (3.80), 436 (3.94); MS (EI), *m/e* (relative intensity) 323, 325 (M^+, 100, 34), 253 (34), 252 (46), 75 (34).

Acknowledgment. This investigation received financial support from the National Health and Medical Research Council of Australia. We thank Dr. D. J. Brown for helpful discussions and Julia MacIntyre for her excellent technical assistance.

Registry No. **2a**, 58137-45-4; **2b**, 112069-51-9; **2c**, 112069-52-0; **2d**, 112069-53-1; **2e**, 112069-54-2; **2f**, 112069-55-3; **2g**, 76896-61-2; **2h**, 112069-56-4; **2i**, 112069-57-5; **2j**, 112069-58-6; **2k**, 112069-59-7; **2l**, 21333-02-8; **3**, 73908-18-6; **4a**, 112069-60-0; **4b**, 112069-61-1; 4c, 112069-62-2; **4d**, 112069-63-3; **4e**, 112069-64-4; **4f**, 112069-65-5; **4g**, 112069-66-6; **4h**, 112069-67-7; **4i**, 112069-68-8; **4j**, 112069-69-9; **4k**, 112069-70-2; **4l**, 65626-88-2; **5**, 112069-71-3; 4-$ClC_6H_4NH_2$, 106-47-8; 2-$ClC_6H_4NH_2$, 95-51-2; 3-$ClC_6H_4NH_2$, 108-42-9; 3-$BrC_6H_4NH_2$, 591-19-5; 4-$BrC_6H_4NH_2$, 106-40-1; 3-$FC_6H_4NH_2$, 372-19-0; 4-$FC_6H_4NH_2$, 371-40-4; 2-Me-4-$ClC_6H_3NH_2$, 95-69-2; 2,4-$Cl_2C_6H_3NH_2$, 554-00-7; 2,5-$Cl_2C_6H_3NH_2$, 95-82-9; 3,5-$Cl_2C_6H_3NH_2$, 626-43-7; 4-$ClC_6H_4CH_2NH_2$, 104-86-9; PhNO, 586-96-9; 6-chloro-3-methyluracil, 4318-56-3; 6-aminouracil, 873-83-6.

Supplementary Material Available: Table listing visible and ultraviolet spectral data for compounds 4a–l (1 page). Ordering information is given on any current masthead page.

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