63·free base, 133445-80-4; 64, 133445-71-3; 64·free base, 133445-81-5; 65, 133445-73-5; 65·free base, 133445-83-7; 66, 129949-86-6; 66·free base, 129949-85-5; 67, 129949-87-7; 68, 129949-91-3; 69, 129949-90-2; 70, 129949-89-9; 71, 129949-96-8; 71·free base, 133445-82-6; 72, 129949-92-4; 72·free base, 129949-93-5; 73, 129949-94-6; 73·free base, 129949-95-7; 2,3-dibromothiophene, 3140-93-0; 2-(2-methoxyethoxy)ethanol, 111-77-3; 2-morpholinoacetamide, 5625-98-9; isobutylamine, 78-81-9; *tert*-butylamine, 75-64-9; methyl bromoacetate, 96-32-2; *N*-(2-methoxyethyl)-*N*-[2-(2-methoxyethoxy)ethyl]amine, 128620-95-1; 2-(2-methoxyethoxy)ethyl-*p*-toluenesulfonate, 50586-80-6; 2-(2-methoxyethoxy)ethyl iodide, 104539-21-1; (2-methoxyethyl)amine, 109-85-3; (2-methoxyethoxy)acetyl chloride, 16024-55-8; 2-(dimethylamino)ethylamine, 108-00-9; 5-[*N*-(3-thia-*n*-butyl)carbamoyl]-thieno[2,3-*b*]thiophene-2-sulfonamide, 133445-79-1; 3-thia-*n*-butylamine, 18542-42-2; 2-morpholinoethylamine, 2038-03-1.

Flavins as Potential Antimalarials. 2. 3-Methyl-10-(substituted-phenyl)flavins

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A series of 3-methyl-10-(substituted-phenyl)flavins was prepared and tested for antimalarial activity against the lethal parasite *Plasmodium vinckei* in mice. Several of these analogues were found to be effective antimalarial agents. A quantitative structure–activity relationship study was undertaken with 44 analogues and no satisfactory relationship could be established.

We have previously found a number of 10-(halophenyl)-3-methylflavins to possess antimalarial activity both in vivo[1,2] against rodent malarias and in vitro against *Plasmodium falciparum*.[2] These agents are potent inhibitors of both human and plasmodial glutathione reductase;[3] inhibition of the latter may account for the antimalarial properties of these agents.[4] In part 1 of this series we examined compounds with only a limited number of 10-phenyl substituents which covered a relatively narrow range of electronic, steric, and lipophilic properties. In the present work we have prepared and tested a number of 3-methyl-10-(substituted-phenyl)flavin analogues with a wide range of physicochemical properties in an attempt to identify the most effective substituent pattern in the 10-phenyl moiety.

Chemistry

The majority of 3-methyl-10-phenylflavins (**2a–ff**; Table II) 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.[5] 6-[4′-(Dimethylamino)anilino]-3-methyluracil (**1o**; Table I) failed to condense with nitrosobenzene in the expected manner. An alternate approach involving the condensation of alloxan with the 2-aminodiphenylamine reduction product of **3** to give the corresponding 10-[4′-(dimethylamino)phenyl]flavin (**4**), followed by methylation, was successful in giving 3-methyl-10-[4′-(dimethylamino)-phenyl]flavin (**2o**; Table II). The 6-anilino-3-methyluracils (**1a–cc**; Table I) were prepared by heating the appropriate aniline with 6-chloro-3-methyluracil and isolated from EtOH and recrystallized from MeOH or acetic acid. This method, described previously,[1,5] called for an excess of the aniline (3 equiv) to be reacted with 6-chloro-3-methyluracil. This procedure, in synthetic terms, is satisfactory; however, in the present work some of the aniline starting materials were either expensive or less readily available synthetically; therefore, we devised a method to help conserve them. The latter consisted of reacting molar equivalents of the chlorouracil and appropriate aniline nucleophile in the pres-

Table I. Physical Properties of 3-Methyl-6-(substituted-anilino)uracils **1**

no.	X	mp, °C	method (% yield)	formula	anal.
1a	H	330–331[a]	B (78)	$C_{11}H_{11}N_3O_2$	C,H,N
1b	2-Me	237–239	B (67)	$C_{12}H_{13}N_3O_2$	C,H,N
1c	3-Me	273–274[b]	B (76)	$C_{12}H_{13}N_3O_2$	C,H,N
1d	4-Me	311–313[c]	A (70)	$C_{12}H_{13}N_3O_2$	C,H,N
1e	2-Et	223–226	B (22)	$C_{13}H_{15}N_3O_2$	C,H,N
1f	3-Et	246–248	A (71)	$C_{13}H_{15}N_3O_2$	C,H,N
1g	4-Et	304–307	B (78)	$C_{13}H_{15}N_3O_2$	C,H,N
1h	4-Bu	268–270	A (62)	$C_{15}H_{19}N_3O_2$	C,H,N
1i	3-CF_3	257–259	B (56)	$C_{12}H_{10}F_3N_3O_2$	C,H,N
1j	4-CF_3	300–302	B (42)	$C_{12}H_{10}F_3N_3O_2$	C,H,N
1k	3-OMe	268–269[d]	A (69)	$C_{12}H_{13}N_3O_3$	C,H,N
1l	4-OMe	310–312[e]	A (75)	$C_{12}H_{13}N_3O_3$	C,H,N
1m	3-SMe	257–259	B (87)	$C_{12}H_{13}N_3O_2S$	C,H,N
1n	4-SMe	327–328	B (80)	$C_{12}H_{13}N_3O_2S$	C,H,N
1o	4-NMe_2	dec 320[f]	A (92)	$C_{13}H_{16}N_4O_2$	C,H,N
1p	4-OH	330–333[g]	B (80)	$C_{11}H_{11}N_3O_3$	C,H,N
1q	4-CN	353–354[h]	B (30)	$C_{12}H_{10}N_4O_2$	C,H,N
1r	2,4-Me_2	290–292	B (59)	$C_{13}H_{15}N_3O_2$	C,H,N
1s	3,4-Me_2	309–312[i]	B (88)	$C_{13}H_{15}N_3O_2$	C,H,N
1t	3,5-Me_2	289–290[j]	B (53)	$C_{13}H_{15}N_3O_2$	C,H,N
1u	3,5-$(CF_3)_2$	305–306	A (81)	$C_{13}H_9F_8N_3O_2$	C,H,N
1v	3,5-$(OMe)_2$	298–299	B (88)	$C_{13}H_{15}N_3O_4$	C,H,N
1w	3-Cl,4-Me	299–300	B (88)	$C_{12}H_{12}ClN_3O_2$	C,H,N
1x	3-Cl,5-Me	303–304	B (60)	$C_{12}H_{12}ClN_3O_2$	C,H,N
1y	4-Cl,3-Me	334–335	B (76)	$C_{12}H_{12}ClN_3O_2$	C,H,N
1z	2-Cl,5-CF_3	311–312	B (68)	$C_{12}H_9ClF_3N_3O_2$	C,H,N
1aa	3-CF_3,4-Cl	283–285	A (75)	$C_{12}H_9ClF_3N_3O_2$	C,H,N
1bb	2-F,4-Cl	335–336	B (42)	$C_{11}H_9ClFN_3O_2$	C,H,N
1cc	3,4-F_2	333–337	B (71)	$C_{11}H_9F_2N_3O_2$	C,H,N

[a] Literature[5] mp 336–338 °C. [b] Literature[5] mp 291 °C. [c] Literature mp 325 °C (Yoneda, F; Tsukuda, K.; Shinozuka, K.; Hirayama, F.; Uekama, K.; Koshiro, A. *Chem. Pharm. Bull.* **1980**, *28*, 3049). [d] Literature mp 276 °C (Grauert, R. W. *Arch. Pharm. Ber. Dtsch. Pharm. Ges.* **1982**, *315*, 949). [e] Literature mp 290–292 °C (Shinkai, S.; Kawanabe, S.; Kawase, A.; Yamaguchi, T.; Manabe, O.; Harada, S.; Nakamura, H.; Kasai, N. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2095). [f] Literature mp >330 °C (Grauert, R. W. *Ibid*). [g] Literature mp >330 °C (Grauert, R. W. *Ibid*). [h] Literature[5] mp 353 °C. [i] Literature mp 309 °C (Grauert, R. W. *Ibid*). [j] Literature mp 275–277 °C (Kurreck, H.; Bock, M.; Bretz, N.; Elsner, M.; Kraus, H.; Lubitz, W.; Müller, F.; Geissler, J.; Kroneck, P. M. H. *J. Am. Chem. Soc.* **1984**, *106*, 737).

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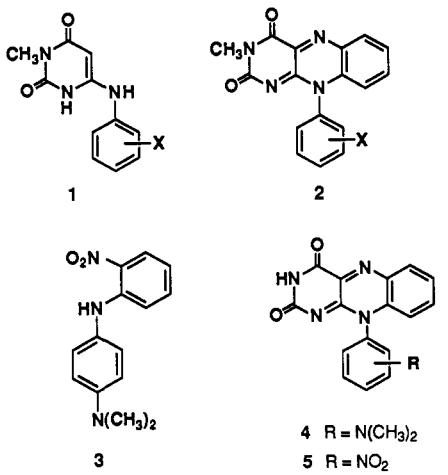
Table II. Physical Properties of 3-Methyl-10-(substituted-phenyl)flavins **2**

no.	X	mp, °C	% yield	formula	anal.
2a	H	>360[a]	35	$C_{17}H_{12}N_4O_2$	C,H,N
2b	2-Me	>360[b]	50	$C_{18}H_{14}N_4O_2$	C,H,N
2c	3-Me	>360[c]	60	$C_{18}H_{14}N_4O_2$	C,H,N
2d	4-Me	367–368	64	$C_{18}H_{14}N_4O_2$	C,H,N
2e	2-Et	270–272[d]	23	$C_{19}H_{16}N_4O_2$	C,H,N
2f	3-Et	277–279	42	$C_{19}H_{16}N_4O_2$	C,H,N
2g	4-Et	318–319	51	$C_{19}H_{16}N_4O_2$	C,H,N
2h	4-Bu	298–300	42	$C_{21}H_{20}N_4O_2$	C,H,N
2i	3-CF_3	346–348	38	$C_{18}H_{11}F_3N_4O_2$	C,H,N
2j	4-CF_3	>360	15	$C_{18}H_{11}F_3N_4O_2$	C,H,N
2k	3-OMe	322–323	48	$C_{18}H_{14}N_4O_3$	C,H,N
2l	4-OMe	353–355[e]	56	$C_{18}H_{14}N_4O_3$	C,H,N
2m	3-SMe	304–305	35	$C_{18}H_{14}N_4O_2S$	C,H,N
2n	4-SMe	>360	38	$C_{18}H_{14}N_4O_2S$	C,H,N
2o	4-NMe_2	326–327	36	$C_{19}H_{17}N_5O_2$	C,H,N
2p	4-OH	>360[f]	24	$C_{17}H_{12}N_4O_3$	C,H,N
2q	4-CN	>360[g]	43	$C_{17}H_{11}N_5O_2$	C,H,N
2r	2,4-Me_2	332–333	56	$C_{19}H_{18}N_4O_2$	C,H,N
2s	3,4-Me_2	333–334	46	$C_{19}H_{18}N_4O_2$	C,H,N
2t	3,5-Me_2	334–335[h]	42	$C_{19}H_{18}N_4O_2$	C,H,N
2u	3,5-$(CF_3)_2$	>360	40	$C_{19}H_{10}F_6N_4O_2$	C,H,N
2v	3,5-$(OMe)_2$	341	45	$C_{19}H_{18}N_4O_4$	C,H,N
2w	3-Cl,4-Me	>360	39	$C_{18}H_{13}ClN_4O_2$	C,H,N
2x	3-Cl,5-Me	>360	34	$C_{18}H_{13}ClN_4O_2$	C,H,N
2y	3-Me,4-Cl	>360	20	$C_{18}H_{13}ClN_4O_2$	C,H,N
2z	2-Cl,5-CF_3	356–357	44	$C_{18}H_{10}ClF_3N_4O_2$	C,H,N
2aa	3-CF_3,4-Cl	>360	44	$C_{18}H_{10}ClF_3N_4O_2$	C,H,N
2bb	2-F,4-Cl	274–275	26	$C_{17}H_{10}ClFN_4O_2$	C,H,N
2cc	3,4-F_2	>360	63	$C_{17}H_{10}F_2N_4O_2$	C,H,N
2dd	4-CO_2H	>360	69	$C_{18}H_{12}N_4O_4$	C,H,N
2ee	4-SO_2Me	334–335	45	$C_{18}H_{14}N_4O_4S$	C,H,N
2ff	3-NO_2	>360	76	$C_{17}H_{11}N_5O_4$	C,H,N

[a] Literature[5] mp >360 °C. [b] Literature mp 372 °C (Main, L.; Kasperek, G. J.; Bruice, T. C. *Biochemistry* **1972**, *11*, 3991). [c] Literature[5] mp 326 °C. [d] Literature mp 262–263 °C (Shinkai, S.; Nakao, H.; Kuwahara, I.; Miyamoto, M.; Yamaguchi, T.; Manabe, O. *J. Chem. Soc. Perkin Trans. 1* **1988**, 313). [e] Literature mp >300 °C (Shinkai, S.; Kawanabe, S.; Kawase, A.; Yamaguchi, T.; Manabe, O.; Harada, S.; Nakamura, H.; Kasai, N. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2095). [f] Literature mp >300 °C (Shinkai, S. et al. *Ibid*). [g] Literature[5] mp >360 °C. [h] Literature[5] mp 347 °C.

ence of two molar equivalents of *N,N*-diethylaniline. This method proved to be very effective, especially in those cases where the aniline nucleophile was a high-melting solid.

3-Nitroaniline did not react with 6-chloro-3-methyluracil under the above conditions; therefore, 3′-nitro analogue **2ff** (Table II) was prepared by methylation of **5**, which was itself prepared by direct nitration of 10-phenylflavin. ^1H NMR of **5** showed that the chemical shifts and splitting patterns for the four benzenoid protons, H6–9, did not change following nitration, while those of the 10-phenyl substituent changed significantly. Thus, nitration occurred on the phenyl substituent and the splitting patterns and chemical shifts were consistent with nitration at the 3′-position since a doublet at 7.4 ppm associated with the 2′- and 6′-protons of the parent, unsubstituted compound was replaced by a singlet (H2′) at 8.4 ppm and a signal centered at 7.95 ppm (H6′). These shifts, as those for H4′ and 5′, are consistent with those induced by *o*- and *p*-nitro substitution, respectively, in benzene rings. Spin decoupling experiments, in which all doublets and multiplets were decoupled, confirmed this assignment.



4′-Hydroxy analogue **2p** was prepared both by the method described above and by cleaving the ether linkage of the 4′-methoxy derivative **2l** with HBr in acetic acid. 4′-Carboxylate **2dd** (Table II) was prepared in good yield by acid-catalyzed hydrolysis of the corresponding nitrile (**2q**; Table II). 4′-Methylsulfonyl analogue **2ee** (Table II) was readily prepared by the action of peroxyacetic acid on the corresponding methylsulfide compound (**2n**; Table II).

Results and Discussion

The results of screening a set of 44 compounds for antimalarial activity are found in Table III. Included in this list are twelve 10-(halophenyl)-3-methylflavins described in part 1 of this series, which were retested at the appropriate doses for direct comparison with the agents prepared in this study.

Several active compounds, notably the 3′-CF_3 and 3′-Cl,5′-CH_3 compounds (**2i** and **2x**; Table III) have been identified in this study. The latter compound was structurally similar to the 3′,5′-Cl_2 (**2qq**) analogue identified previously[1] as the most active compound in that series. 3′,5′-$(CH_3)_2$ derivative **2t** was also very active in this screen, as were several others at higher doses.

A trend immediately discernable from Table III is that compounds with 2′-substituents are toxic compared with their 3′- and 4′-substituted analogues, for example, compare 2′-methyl derivative **2b** with its 3′- and 4′-methyl analogues **2c** and **2d**, respectively, and similarly ethyl-substituted compounds **2e** vs **2f** and **2g** (Table III). This trend was also found with disubstituted compounds; for example, compounds **2r** and **2z** are toxic compared with their respective analogues **2s** and **2aa** (Table III).

In comparing the disubstituted derivatives the 3′,5′-disubstituted compounds are more active than their 3′,4′-disubstituted structural isomers. This is especially discernable, for example, where 3′-Cl,5′-Me analogue **2x** is considerably more active than either of its 3′,4′-substituted phenyl structural isomers (**2w** and **2y**). 3′,5′-Dimethyl derivative **2t** is also more active than its 3′,4′-disubstituted isomer (**2s**).

In part 1 of this series we demonstrated a requirement for the 10-phenyl group and the importance of its substitution pattern for antimalarial activity in an initial series of 10-(halophenyl)-3-methylflavins. In the present work we have attempted to identify the functional groups and the substitution pattern(s) in the phenyl group which impart optimum activity in the parent series prior to making changes elsewhere in the molecule. In order to limit the number of compounds to be synthesized we initially

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Table III. Parenteral Antimalarial Activity of 3-Methyl-10-(substituted-phenyl)flavins against *P. vinckei vinckei* in Mice[a]

drug[b]	X	percent cured (increase in mean survival days) at dose, mg/kg								
		10	15	20	25	30	40	50	60	70
2a	H	0 (0)				0 (0)		0 (0.3)	20 (0.5)	60 (1.0)
2b	2-Me	0 (−0.2)		0 (−1.0)[c]		0 (−2.0)[c]				
2c	3-Me	0 (0.8)	0 (0)	0 (0.5)	0 (0)	0 (0)		0 (0.5)		0 (0.3)
2d	4-Me	0 (0)				0 (0.2)		0 (0.3)		0 (0)
2e	2-Et	0 (0)		0 (−2.0)[c]	0 (−2.0)[c]					
2f	3-Et		0 (0)	0 (0)	0 (0)	0 (0)				0 (0.3)
2g	4-Et	0 (0.2)		0 (0.3)		0 (0)				0 (−0.4)
2h	4-Bu	0 (0)	0 (0)	0 (0)	0 (0.1)	0 (0)		0 (0)		0 (0.5)
2i	3-CF_3	33 (1.4)	100	100	100	100	100	100	40 (10)	0 (7.5)[c]
2j	4-CF_3	0 (0)		0 (0)		80 (2.0)		100	100	100
2k	3-OMe	0 (0)		0 (0)		0 (0)				0 (0)
2l	4-OMe	0 (0)				0 (0.4)	20 (1.0)	80 (2.0)	80 (1.0)	60 (−0.5)[c]
2m	3-SMe	0 (0.2)		0 (0)		0 (0)				0 (0)
2n	4-SMe	0 (0.2)		0 (0.5)		0 (0.5)				0 (0)
2o	4-NMe_2	0 (0)				0 (0)				0 (0)
2p	4-OH	0 (0)				0 (0.2)				0 (0)
2q	4-CN	0 (0)				0 (0)				0 (0)
2r	2,4-Me_2	0 (0.4)				20 (1.3)	40 (−1.7)[c]	0 (−2.0)[c]		0 (−2.0)[c]
2s	3,4-Me_2	0 (0.3)		0 (0.2)		0 (0.8)		20 (0.3)	60 (0.5)	100
2t	3,5-Me_2	0 (0)	60 (1.0)	100	100	100	100	60 (5.5)	0 (0.4)[c]	
2u	3,5-$(CF_3)_2$	0 (0)		0 (0)		0 (0)		0 (0)		0 (0)
2v	3,5-$(OMe)_2$	0 (0)				0 (0)		0 (0)	20 (3.0)	100
2w	3-Cl,4-Me	0 (0)				0 (0)				0 (0)
2x	3-Cl,5-Me	0 (0.2)	60 (0.5)	80 (1.0)	100	100	100	100		100
2y	4-Cl,3-Me	0 (0)				0 (0)	0 (0.3)	25 (0.3)	100	80 (2.0)
2z	2-Cl,5-CF_3	0 (0)	0 (0.2)	0 (0)	0 (0)[c]	0 (−0.4)[c]				
2aa	3-CF_3,4-Cl	0 (0)	0 (0)	0 (0)		0 (0)				0 (0.2)
2bb	2-F,4-Cl	0 (1.0)		0 (4.0)		0 (−2.0)[c]				
2cc	3,4-F_2	0 (0)		0 (−0.5)		0 (0)				0 (−1.0)[c]
2dd	4-CO_2H	0 (0)				0 (0.2)				0 (−0.2)
2ee	4-SO_2Me	0 (0)				0 (0.2)	0 (−0.5)	0 (−0.8)[c]		0 (−2.0)[c]
2ff	3-NO_2	0 (0)				0 (0)			0 (1.0)	0 (−0.5)
2gg	3-F[d]	0 (0.2)	0 (0.2)	0 (0)	0 (0)	0 (0.2)	0 (0.8)	20 (0)		0 (−1.0)[c]
2hh	4-F[d]	0 (0)	0 (0)	0 (13.4)		80 (0)	80 (0)	20 (−0.5)		0 (−0.6)[c]
2ii	2-Cl[d]	0 (0)				0 (−0.3)[c]				0 (−2.0)[c]
2jj	3-Cl[d]	0 (0)				0 (0)		0 (0.4)	50 (0.5)	100
2kk	4-Cl[d]	20 (0.3)	80 (4.0)	100	100	100	100	20 (−0.8)[c]		0 (−1.0)[c]
2ll	3-Br[d]	0 (0)	0 (0)			0 (0)	0 (0)	80 (1.0)	80 (−1.0)	100
2mm	4-Br[d]	0 (1.3)	100	100	100	100		40 (9.0)		0 (7.5)
2nn	2,4-Cl_2[d]	0 (1.7)	0 (0.5)		0 (−1.2)[c]	0 (−1.0)[c]				
2oo	2,5-Cl_2[d]	0 (0)				0 (0)				0 (−0.6)[c]
2pp	3,4-Cl_2[d]	0 (0)				0 (0)		0 (0)		0 (0)
2qq	3,5-Cl_2[d]	100	100	100	100	100	100			60 (16.5)
2rr	4-Cl,2-Me[d]	0 (0)		0 (1.2)	0 (−1.5)[c]	0 (−2.0)[c]				

[a] Animals were considered cured if still living 60 days posttreatment with a single intraperitoneal injection in olive oil (100 μL). [b] For comparison at 10 mg/kg chloroquine diphosphate gave 80 (2.0) and cured all animals treated at doses of 15 mg/kg or higher. [c] Toxic. [d] Compound described in ref 1 retested over appropriate dose range.

planned our syntheses around the non-computer-assisted "batchwise" analysis of small groups of compounds as described by Topliss.[6] This approach seemed especially suitable in the present case as we had previously prepared and tested several analogues in both the "initial" and "second compound" groups. For the present series, however, this method was found to be ineffective as a predictive tool. Since we had prepared and tested a large number of compounds which covered a good range of lipophilic, steric, and electronic properties, we decided to perform a more comprehensive statistical treatment of our results.

"Hansch" methodology[7] was used on a set of 17 compounds for which ED_{40} values, based on percent parasitemia 48 h following treatment, had been determined (Table IV). The individual values were estimated by interpolation from linear-regression analysis of the biological response (log $(p/(100 - p))$, where p is the para-

Table IV. ED_{40} Values of Some Active 3-Methyl-10-(substituted-phenyl)flavins[a]

drug	X	ED_{40}, mmol/kg × 10^{-3}	95% confidence limits	
			lower	upper
2mm	4-Br	38.4	35.4	40.9
2kk	4-Cl	38.8	35.5	41.6
2qq	3,5-Cl_2	40.2	36.0	44.1
2i	3-CF_3	79.3	73.1	86.1
2x	3-Cl,5-Me	85.7	80.2	92.6
2hh	4-F	103	95.0	112
2t	3,5-$(Me)_2$	105	94.2	118
2j	4-CF_3	135	124	146
2l	4-OMe	138	133	143
2ll	3-Br	148	138	162
2y	4-Cl,3-Me	182	176	195
2s	3,4-$(Me)_2$	210	205	216
2v	3,5-$(OMe)_2$	219	210	229
2jj	3-Cl	229	221	242
2a	H	248	235	290
2g	4-Et	281	271	292
2w	3-Cl,4-Me	456	363	582

[a] Effective dose in mmol/kg required to obtain a parasitemia of 40% 48 h after treatment.

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sitemia of individual mice 48 h after treatment, versus dose. The regression was restricted to the linear portion of the plot corresponding to the change in activity. In this study no significant relationships between the ED_{40} values and physicochemical properties could be found. This analysis was restricted to only those compounds for which ED_{40} values could be obtained; thus, the physicochemical "space" covered was reduced when compared to that covered by the entire group of 44 compounds screened for activity. Logistic regression[8] is a statistical analysis that allows inclusion of all members of a series tested by considering the response as binary, in this case the biological response was entered as either active (1) or inactive (0). This method is similar to previously used methods of discriminant analysis[9,10] in that it allows the use of qualitative biological responses. Following a complete analysis it was found that none of the terms were significant, thus no equation found was superior to the null model.

Conclusions

The 10-phenyl substituents examined in this study covered a wide range of lipophilic, steric, and electronic properties, and despite being unable to establish a quantitative structure–activity relationship, several new active compounds have been identified. Ariëns[11] has outlined eight factors which can account for an apparent lack of a structure–activity relationship for a given series of agents. Several of these factors relate specifically to biological response data derived from in vivo test systems. We believe this to be the case in the present study since most of the compounds examined so far in this series demonstrate a wide range of activity between individual members in mice but are essentially equipotent against *P. falciparum* in culture.[12]

The present study has identified several 10-phenyl substituents and substitution patterns which impart considerable activity to this series of 3-methyl-10-phenylflavins. These findings can be utilized in future studies where structural changes will be made elsewhere in the molecule, including the benzenoid ring-3 of the flavin and the 3-N substituent. A few promising leads in the latter series have recently been identified.[4]

Experimental Section

Melting points are uncorrected. Analyses were performed by the Analytical Service Unit of the Australian National University and C, H, N values were within ±0.4% of the theoretical values. ^1H nuclear magnetic resonance (^1H NMR) spectra were recorded on either a JEOL 90 MHz (FX 90) or a Varian 200 MHz (XL 200) spectrometer and are expressed in parts per million (δ) downfield from an internal tetramethylsilane standard. Ultraviolet spectra were recorded on a Varian DMS 100 UV–visible spectrophotometer. Nitrosobenzene and anilines, unless indicated otherwise, were obtained from Aldrich Chemical Co.

Antimalarial Screening in Mice. The 3-methyl-10-(substituted-phenyl)flavins were tested against a normal drug-sensitive strain of *P. vinckei vinckei* (strain V52, from F. E. G. Cox, Kings College, London, U.K.) which had been passaged several times before use. Female, specific-pathogen free, CBA/CaH mice (6–8 weeks old) were used in all in vivo experiments. Infections were initiated by intraperitoneal (ip) injection of 5×10^5 parasitized red blood cells and monitored by examining stained (Diff-Quik, Australian Hospital Supply) 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 parenteral administration the powdered drugs were suspended in olive oil and administered as a single dose by ip injection (100 μL) on day 6. Control animals received olive oil alone. These results are summarized in Tables III and IV.

6-Anilino-3-methyluracils (1a–cc). These compounds were prepared either as described previously[1] by heating 6-chloro-3-methyluracil with 3 equiv of the required aniline (method A) or by the following modified procedure (method B): a mixture of 6-chloro-3-methyluracil (1.6 g, 10 mmol), the appropriate aniline (10 mmol), *N,N*-diethylaniline (3.0 g, 3.2 mL, 20 mmol), and acetic acid (0.5 mL) was heated at 190 °C for 25 min, cooled briefly, poured into EtOH (25 mL), and stirred until crystallization was complete. The solid was filtered off, washed with ether, and recrystallized from either MeOH or acetic acid and dried to give **1a–cc** (Table I). Analytical samples were recrystallized from MeOH.

3-Methyl-10-(substituted-phenyl)flavins (2a–n, 2p–cc). The appropriate 6-anilino-3-methyluracil (1) (10 mmol) and nitrosobenzene (3.2 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 EtOH (10 mL) added. After crystallization was complete the solid was filtered off, washed with EtOH and ether, and recrystallized from MeOH or acetic acid to give compounds **2a–n, 2p–cc** (Table II). Analytical samples were prepared from MeOH.

10-(4′-Hydroxyphenyl)-3-methylflavin (2p). Compound **2l** (0.6 g, 1.8 mmol) was refluxed with HBr in acetic acid (27% w/v, 30 mL) for 12 h, additional HBr in acetic acid (10 mL) was added and refluxing continued for a further 8 h. The mixture was taken to dryness under reduced pressure and the product recrystallized from MeOH to give **2p** (0.4 g, 69%) identical (NMR, UV, TLC) with that prepared by method A above.

10-(4′-Carboxyphenyl)-3-methylflavin (2dd). Compound **2q** (0.5 g, 1.6 mmol) was refluxed in a mixture of acetic acid (100 mL) and 70% H_2SO_4 (100 mL) for 2 h. The cooled mixture was poured into ice water (200 mL) and the product precipitated with solid Na_2CO_3. The solid was filtered off, washed with water, and recrystallized from acetic acid to give **2dd** (Table II). The analytical sample was recrystallized from acetic acid: UV λ_{max} (log ϵ) (EtOH) 269 (4.51), 336 (3.87), 440 (3.97); MS (EI) m/e (relative intensity) 348 (M, 38), 347 (100), 290 (28), 263 (35). Anal. ($C_{18}H_{12}N_4O_4$) C, H, N.

3-Methyl-10-[4′-(methylsulfonyl)phenyl]flavin (2ee). Compound **2n** (0.2 g, 0.57 mmol) was dissolved in acetic acid (50 mL) and cooled to <5 °C. To this solution was added 30% w/v H_2O_2 (0.26 g, 2.3 mmol), dropwise with stirring over 1 h. The mixture was allowed to come to room temperature and stirred for an additional 18 h. The acetic acid was evaporated off under a stream of nitrogen in an evaporating dish and the solid recrystallized from MeOH to give **2ee** (Table II). The analytical sample was recrystallized from 2-propanol: UV λ_{max} (log ϵ) (EtOH) 267 (4.50), 335 (3.88), 438 (3.99); MS (CI) m/e (relative intensity) 383 (M + 1, 7) 367 (91), 351 (100), 229 (47). Anal. ($C_{18}H_{14}N_4O_4S$) C, H, N.

4′-(Dimethylamino)-2-nitrodiphenylamine (3). A mixture of 2-chloro-1-nitrobenzene (25 g, 0.16 mol) and sodium acetate (25 g, 0.3 mol) was heated, with stirring, at 170–180 °C under a nitrogen atmosphere while *N,N*-dimethyl-1,4-phenylenediamine (22.0 g, 0.16 mol) was added portionwise over 7 h. The resulting black tar was solubilized with a small volume of EtOH and made acidic with 1 M HCl. The solution was steam distilled for ca. 2.5 h to remove unreacted 2-chloro-1-nitrobenzene. The residue was made basic with aqueous ammonia and the resulting oil separated, washed with water (3 × 20 mL), and extracted several times with warm ether. The ether extracts were combined, dried over Na_2SO_4, and filtered and the volatiles removed under reduced pressure. The resulting oily residue was dissolved in a small volume of hot EtOH, treated with charcoal, filtered, reduced in volume, and chilled to give red crystals of **3** (2.1 g, 5.1%). The air- and light-sensitive analytical sample was recrystallized from

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2-propanol: mp 97–98 °C; MS (EI) m/e (relative intensity) 257 (M, 100), 223 (65), 209 (31), 167 (32), 136 (42). Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

10-[4′-(Dimethylamino)phenyl]flavin (4). Diphenylamine **3** (1.2 g, 4.7 mmol) was added to a nitrogen-saturated solution of stannous chloride (5.3 g, 23 mmol) in concentrated HCl (10 mL) and acetic acid (50 mL) at 60 °C with stirring. A stream of nitrogen was passed through the solution continuously while stirring was continued for 1.3 h. The reaction was cooled and made basic with nitrogen saturated 10 M NaOH solution and diluted with nitrogen-saturated water to dissolve the remaining tin salts. The resulting oil was extracted into $CHCl_3$ and dried (Na_2SO_4), the $CHCl_3$ removed under reduced pressure, and the oil (1.0 g, 4.5 mmol) dissolved in acetic acid (100 mL). To this solution was added alloxan tetrahydrate (1.0 g, 4.5 mmol) and boric acid (0.3 g, 4.8 mmol) and the mixture heated at 60 °C for 1 h under a nitrogen atmosphere. After evaporation to dryness the residue was washed with a small volume of water and ether and recrystallized from MeOH to give **4** (0.4 g, 23%). The analytical sample was recrystallized from MeOH: mp >330 °C dec; UV λ_{max} (log ϵ) (EtOH) 265 (4.63), 326 (3.82), 439 (3.98); ^{1}H NMR (DMSO-d_6) δ 8.16 (1 H,d,$J_{6,7}$ = 7.2 Hz, H6), 7.53–7.82 (2 H, m, H7,8), 7.17 (2 H, d, J = 7.7 Hz), 6.93 (3 H, d, J = 7.7 Hz), 3.02 (6 H, s, NMe_2); MS (EI) m/e (relative intensity) 333 (M, 95), 332 (71), 261 (100), 247 (25), 131 (33), 130 (26). Anal. ($C_{18}H_{15}N_5O_2$) C, H, N.

10-[4′-(Dimethylamino)phenyl]-3-methylflavin (2o). To a solution of **4** (0.2 g, 0.56 mmol) and K_2CO_3 (0.73 g, 5.6 mmol) in DMF (120 mL) was added MeI (0.95 g, 6.7 mmol). The mixture was heated and stirred at 50 °C for 1 h, cooled, and filtered and the filtrate added to $CHCl_3$ (400 mL). This was washed with water (5 × 200 mL), dried (Na_2SO_4), filtered, and taken to dryness under reduced pressure. The solid was washed with ether and recrystallized from MeOH to give **2o** (Table III): UV λ_{max} (log ϵ) (EtOH) 265 (4.69), 326 (3.86), 437 (3.95); ^{1}H NMR (DMSO-d_6) δ 6.8–8.3 (8 H, m, aromatic), 3.25 (3 H, s, NMe), 3.02 (6 H, s, NMe_2); MS (EI) m/e (relative intensity) 347 (M, 100), 262 (37), 261 (66), 131 (46).

10-(3′-Nitrophenyl)flavin (5). To a solution of 10-phenylflavin[13] (1.5 g, 5 mmol) in concentrated sulfuric acid (10 mL) was added fuming nitric acid (d = 1.5, 0.34 g, 5.5 mmol). The solution was heated to 130 °C for 1.5 h, allowed to cool, poured onto ice (100 g), and adjusted to pH 6 with concentrated ammonia solution. The precipitate was filtered off, washed with EtOH, and recrystallized from MeOH to give **5** (0.6 g, 34%): mp 253 °C dec; ^{1}H NMR (DMSO-d_6) δ 6.86 (d, $J_{8,9}$ = 8.0 Hz, H9), 7.60–7.78 (m, H7,8), 7.92–8.08 (m, H5′,6′), 8.21 (d, $J_{6,7}$ = 8.0 Hz, H6), 8.42 (s, H 2′), 8.53 (d, $J_{4',5'}$ = 8.0 Hz, H4′); MS (EI) m/e (relative intensity) 335 (M, 34) 334 (100), 292 (16), 291 (22), 290 (21), 289 (76), 264 (19), 263 (30). Anal. ($C_{16}H_9N_5O_4$) C, H, N.

3-Methyl-10-(3′-nitrophenyl)flavin (2ff). To a solution of **5** (1.2 g, 3.5 mmol) in dimethylformamide (250 mL) was added powdered potassium carbonate (4.7 g, 35 mmol) followed by MeI (0.65 g, 4.6 mmol). The mixture was heated and stirred at 50–55 °C for 1 h, cooled, and filtered and the filtrate added to chloroform (400 mL). This was washed with water (5 × 200 mL), dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. The solid residue was washed with ether and recrystallized from MeOH to give **2ff** (Table II): ^{1}H NMR (DMSO-d_6) δ 6.9–8.3 (8 H, m, aromatic), 3.26 (3 H, s, NMe).

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Registry No. **1a**, 7269-95-6; **1aa**, 133373-71-4; **1b**, 133373-55-4; **1bb**, 133373-72-5; **1c**, 58137-46-5; **1cc**, 133373-73-6; **1d**, 76896-60-1; **1e**, 133373-56-5; **1f**, 133373-57-6; **1g**, 133373-58-7; **1h**, 133373-59-8; **1i**, 133373-60-1; **1j**, 133373-61-2; **1k**, 59776-23-7; **1l**, 57712-81-9; **1m**, 133373-62-3; **1n**, 133373-63-4; **1o**, 83983-31-7; **1p**, 83983-30-6; **1q**, 65626-84-8; **1r**, 133373-64-5; **1s**, 59776-24-8; **1t**, 88200-81-1; **1u**, 133373-65-6; **1v**, 133373-66-7; **1w**, 133373-67-8; **1x**, 133373-68-9; **1y**, 133373-69-0; **1z**, 133373-70-3; **2a**, 35804-39-8; **2aa**, 117284-94-3; **2b**, 35919-98-3; **2bb**, 133373-82-7; **2c**, 65626-85-9; **2cc**, 133373-83-8; **2d**, 133373-74-7; **2dd**, 133373-84-9; **2e**, 104870-02-2; **2ee**, 133373-85-0; **2f**, 117284-98-7; **2ff**, 133373-86-1; **2g**, 117285-05-9; **2gg**, 112069-64-4; **2h**, 117284-96-5; **2hh**, 112069-65-5; **2i**, 117284-95-4; **2ii**, 112069-60-0; **2j**, 117285-01-5; **2jj**, 112069-61-1; **2k**, 117284-97-6; **2kk**, 65626-87-1; **2l**, 95353-17-6; **2ll**, 112069-62-2; **2m**, 117285-02-6; **2mm**, 112069-63-3; **2n**, 117285-03-7; **2nn**, 112069-67-7; **2o**, 133373-75-8; **2oo**, 112069-68-8; **2p**, 119100-14-0; **2pp**, 65626-88-2; **2q**, 65626-89-3; **2qq**, 112069-69-9; **2r**, 117285-04-8; **2rr**, 112069-66-6; **2s**, 65626-86-0; **2t**, 117284-99-8; **2u**, 133373-76-9; **2v**, 133373-77-0; **2w**, 133373-78-1; **2x**, 133373-79-2; **2y**, 133373-80-5; **2z**, 133373-81-6; **3**, 129880-96-2; **4**, 133373-87-2; **5**, 133373-88-3; 6-chloro-3-methyluracil, 4318-56-3; nitrosobenzene, 586-96-9; 2-chloro-1-nitrobenzene, 88-73-3; *N*,*N*-dimethyl-*p*-phenylenediamine, 99-98-9; 2-aminodiphenylamine, 534-85-0; alloxan, 50-71-5; 10-flavin, 6851-14-5.

Supplementary Material Available: UV–vis λ_{max} and log ϵ data for **2a–ff** (1 page). Ordering information is given on any current masthead page.

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