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3-Methyl-8-(substituted phenyl)-pteridine-2,4(3*H*,8*H*)-diones as Potential Antimalarials

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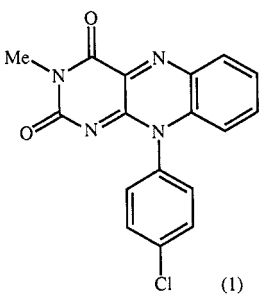
Abstract

A series of 6,7,8-substituted 3-methylpteridine-2,4(3*H*,8*H*)-diones was synthesized by condensation of 5-amino-6-(substituted anilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione with α-dicarbonyl reagents. These compounds were tested for antimalarial activity against the human parasite *Plasmodium falciparum* in culture, and lethal *Plasmodium vinckei vinckei* in mice. The 6,7-unsubstituted pteridinediones were active against cultured *P. falciparum;* however, none of the series was active against *P. vinckei vinckei* in mice.

Introduction

We have shown 10-(4′-chlorophenyl)-3-methylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione [10-(4′-chlorophenyl)-3-methylflavin] (1) to be a potent and novel antimalarial *in vitro* against the human parasite *Plasmodium falciparum* and against *Plasmodium vinckei vinckei* in mice.[1] In an investigation of the structure activity relationships of these agents,[2,3] a series of 6,7,8-substituted 3-methylpteridine-2,4(3*H*,8*H*)-diones (3a–f) was synthesized and tested to determine the necessity of the benzenoid ring (ring 3) of the benzo[*g*]pteridine-2,4(3*H*,10*H*)-diones for antimalarial activity. The substituents 4′-chloro and 3′,5′-dimethyl, in the 8-phenyl ring of the pteridinediones, were selected because of their previously demonstrated beneficial effect on biological activity in the benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione series.[2,3]



(1)

The present study showed that ring 3 of the parent series is necessary for *in vivo* activity. Additionally only the 6,7-unsubstituted pteridinediones (3a,b) were active in the *in vitro* screen.

[1] Cowden, W. B., Butcher, G. A., Hunt, N. H., Clark, I. A., and Yoneda, F., *Am. J. Trop. Med. Hyg.*, 1987, **37**, 495.
[2] Cowden, W. B., Clark, I. A., and Hunt, N. H., *J. Med. Chem.*, 1988, **31**, 799.
[3] Cowden, W. B., and Clark, I. A., *Trans. R. Soc. Trop. Med. Hyg.*, 1987, **81**, 533.

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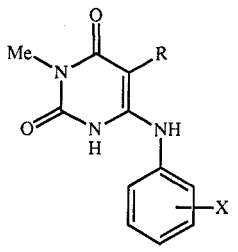
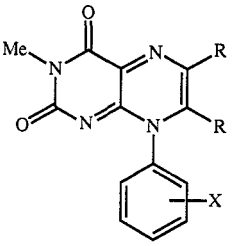
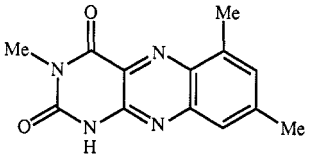


	R	X
(2a)	H	4'-Cl
(2b)	H	3',5'-Me_2
(2c)	NO	4'-Cl
(2d)	NO	3',5'-Me_2
(2e)	NH_2	4'-Cl
(2f)	NH_2	3',5'-Me_2



	R	X
(3a)	H	4'-Cl
(3b)	H	3',5'-Me_2
(3c)	Me	4'-Cl
(3d)	Me	3',5'-Me_2
(3e)	Ph	4'-Cl
(3f)	Ph	3',5'-Me_2



(4)

Results and Discussion

Synthesis

The 6-anilino-3-methylpyrimidine-2,4(1*H*,3*H*)-diones (2a,b) were formed by heating an intimate mixture of 3 equiv. of the appropriate aniline with 6-chloro-3-methylpyrimidine-2,4(1*H*,3*H*)-dione.[2] Nitrosation of the resulting 6-(substituted anilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-diones (2a,b) in trifluoroacetic acid readily gave the corresponding 6-(substituted anilino)-3-methyl-5-nitrosopyrimidine-2,4(1*H*,3*H*)-diones (2c,d) as trifluoroacetic acid salts (not characterized) which were easily reduced with sodium dithionite to the corresponding 5-amino-6-(substituted anilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-diones (2e,f). Compounds similar to the 5-nitroso (2c,d) and 5-amino (2e,f) pyrimidine-2,4(1*H*,3*H*)-diones have been reported to easily convert, through ring closure involving the 5-nitroso/amino group and C2′ of the anilino substituent, into the corresponding benzo[*g*]pteridinediones or their *N*-oxides.[4–6] Therefore, care was taken not to allow these compounds to be heated during their preparation. An example of this unwanted reaction occurred when attempts to purify 5-amino-6-(3′,5′-dimethylanilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (2f) by recrystallization led to the formation of 3,6,8-trimethylbenzo[*g*]pteridine-2,4(1*H*,3*H*)dione (4). Its structure was confirmed by preparing it in a similar manner to that of Goldner *et al.*[4] by briefly heating 6-(3′,5′-dimethylanilino)-3-methyl-5-nitrosopyrimidine-2,4(1*H*,3*H*)-dione (2d) in acetic acid. The final synthetic step in the preparation of the 8- or 6,7,8-substituted 3-methyl pteridine-2,4(3*H*,8*H*)-diones (3a–f) involved the Gabriel and Colman condensation of the 5-amino compounds (2e,f) with α-dicarbonyl reagents. This gave the 6,7,8-substituted 3-methylpteridine-2,4(3*H*,8*H*)-diones in reasonable yields.

Ram *et al.*[7] have made similar compounds using an alternative reaction sequence which we found to be less convenient.

[4] Goldner, H., Dietz, G., and Carstens, E., *Justus Liebigs Ann. Chem.*, 1966, **694**, 142.
[5] Taylor, E. C., Sowinski, F., Yee, T., and Yoneda, F., *J. Am. Chem. Soc.*, 1967, **89**, 3369.
[6] Sakuma, Y., Nagamatsu, T., and Yoneda, F., *J. Chem. Soc., Chem. Commun.*, 1975, 977.
[7] Ram, V. J., Knappe, W. R., and Pfleiderer, W., *Justus Liebigs Ann. Chem.*, 1982, 762.

Biological Testing

The two antimalarial screens used for this series of compounds were inhibition *in vitro* of the human parasite *P. falciparum,* and inhibition in mice of the lethal *P. vinckei vinckei.*

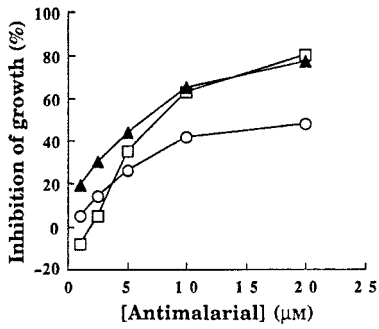


Fig. 1. Growth suppression of *P. falciparum* after 48 h incubation with inhibitors: □ (3a); ○ (3b); and ▲ (1).

Fig. 1 shows that the compounds which were unsubstituted in positions 6 and 7 (3a,b) showed activity in the *in vitro* screen over the same concentration range as the original lead compound benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (1). The apparent lack of increased activity of (3b) at higher concentrations was due to its poor solubility in the testing medium. The other four pteridinediones (3c–f) containing 6,7-dimethyl or 6,7-diphenyl substituents failed to show any activity in the dose range tested. This suggests that the presence of bulky groups in both the 6 and 7 positions abolishes antimalarial activity in the 6,7,8-substituted 3-methylpteridine-2,4(3*H*,8*H*)-diones.

The complete series of pteridinediones (3a–f) and 3,6,8-trimethylbenzo[*g*]-pteridine-2,4(1*H*,3*H*)-dione (4) were tested *in vivo* and found to be inactive in the same dose range over which the 10-(4′-chlorophenyl)-3-methylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione (1) had shown activity. In light of their *in vitro* activity compounds (3a,b) were tested at higher doses in mice where still no activity was seen. This inactivity of the pteridinediones, when compared with the high activity of the 10-(substituted phenyl)-3-methylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-diones, such as (1), could possibly be attributed to pharmacokinetic effects brought about by the absence of the benzoid ring 3 in the pteridine series. The results of the two types of antimalarial screens highlight the significance of this structure activity relationship. Further study on the structure activity relationships of the lead benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione compounds is in progress.

Experimental

Antimalarial Activity

The growth inhibition of *P. falciparum* (FC27, a Papua New Guinea stain maintained *in vitro* over several years) by the pteridinediones (3a–f) was determined by ^{3}H-hypoxanthine incorporation over 48 h incubation as in Cowden *et al.*[1] The inhibition by the lead compound (1) was determined under the same conditions for comparison.

In vivo antimalarial activity was screened by intraperitoneal injection of the pteridinediones (3a–f) and 3,6,8-trimethylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione (4) into mice infected with *P.*

vinckei vinckei (V52) as described previously.[2] The percentage of animals cured, in groups of five mice, was used to detect activity. All compounds were tested at doses of 10, 30 and 70 mg/kg, and compounds (3a,b) were also tested at 200 mg/kg.

General Instrumental

Melting points are uncorrected. Analyses were performed by the Australian National University Analytical Services Unit. ^{1}H nuclear magnetic resonance spectra were recorded on a Varian XL-200 spectrometer. Chemical shifts are expressed as ppm downfield from tetramethylsilane. Mass spectra were recorded on an Incos data system attached to a VG MicroMass 7070F spectrometer at 70 eV.

*6-(3′,5′-Dimethylanilino)-3-methylpyrimidine-2,4(1*H,3H*)-dione (2b)*

A mixture of 6-chloro-3-methylpyrimidine-2,4(1*H*,3*H*)-dione[8] (1·6 g, 10 mmol), 3,5-dimethylaniline (3·6 g, 30 mmol) and acetic acid (0·5 ml) was heated at 190° for 20 min, cooled briefly and poured into methanol (50 ml), and stirred until crystallization was complete. The solid was filtered off, washed with ether, and recrystallized from acetic acid to give (2*b*) (1·5 g, 61%), m.p. 289–290° (Found: C, 63·7; H, 6·3; N, 17·2. $C_{13}H_{15}N_3O_2$ requires C, 63·7; H, 6·2; N, 17·1%). *m/z* (rel. int.) 246 (15%), 245 M (90), 244 (18), 187 (23), 160 (36), 159 (22). ^{1}H n.m.r. (CD_3SOCD_3) δ 2·24, s, (CH_3)$_2$C$_6$H$_3$; 3·05, s, CH_3N; 4·84, s, H5; 6·79, s, H2′,4′,6′.

*5-Amino-6-(4′-chloroanilino)-3-methylpyrimidine-2,4(1*H,3H*)-dione (2e)*

To a stirred cooled solution of 6-(4′-chloroanilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione[2] (7·7 g, 30 mmol) in trifluoroacetic acid (50 ml) was added a sodium nitrite solution (3·2 g, 46 mmol) in water (20 ml) dropwise over 15 min. After stirring for an additional 10 min the resulting 6-(4′-chloroanilino)-3-methyl-5-nitrosopyrimidine-2,4(1*H*,3*H*)-dione trifluoroacetic acid salt was filtered off, washed with ether, dried, pulverized, and suspended in a solution of 1 M NaOH and methanol (4 : 1, 250 ml). Sodium dithionite was added with stirring until the suspension's red colour disappeared. The product was filtered off, washed with water and ether, and recrystallized from methanol to give (2*e*) as a white powder (4·6 g, 56%), m.p. 230–232° (Found: C, 49·6; H, 4·2; N, 20·9. $C_{11}H_{11}ClN_4O_2$ requires C, 49·5; H, 4·2; N, 21·0%). *m/z* (rel. int.) 268 (32%), 267 (15), 266 M (100), 154 (16), 138 (26). ^{1}H n.m.r. (CD_3SOCD_3) δ 3·15, s, CH_3N; 6·80, d, $J_{2',3'}$ 8·9 Hz, H2′,6′; 7·25, d, $J_{2',3'}$ 8·9 Hz, H3′,5′.

*5-Amino-6-(3′,5′-dimethylanilino)-3-methylpyrimidine-2,4(1*H,3H*)-dione (2f)*

6-(3′,5′-Dimethylanilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (2b) (7·35 g, 30 mmol) was treated as for (2e) above to give (2f) (5·2 g, crude yield 67%).

*3,6,8-Trimethylbenzo[g]pteridine-2,4(1*H,3H*)-dione (4)*

(A) Recrystallization of the crude 5-amino-6-(3′,5′-dimethylanilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (2f) (0·5 g, 2 mmol) from methanol gave 3,6,8-trimethylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione *(4)* (0·3 g, 60%), m.p. 310–312° (Found: C, 61·2; H, 5·0; N, 21·9. $C_{13}H_{12}N_4O_2$ requires C, 60·9; H, 4·7; N, 21·9%). *m/z* (rel. int.) 257 (15%), 256 M (100), 199 (20), 171 (40), 156 (19). ^{1}H n.m.r. (CD_3SOCD_3) δ 2·51, s, 8-CH_3; 2·70, s, 6-CH_3; 3·30, s, CH_3N; 7·47, s, 1 aromatic H; 7·51, s, 1 aromatic H.

(B) An aqueous sodium nitrite solution (1·7 g, 25 mmol, in 10 ml of water) was added to a hot (100°) stirred solution of 6-(3′,5′-dimethylanilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (2b) (1·2 g, 5 mmol) in glacial acetic acid (50 ml); the mixture went fleetingly red before becoming yellow. On cooling crystals formed, which were filtered off, and washed with water and methanol. Recrystallization from glacial acetic acid gave light yellow crystals of

[8] Nübel, G., and Pfleiderer, W., *Chem. Ber.*, 1962, **95**, 1605.

(4) (0·9 g, 70%) identical with the compound from (A) by m.p., mass spectrum and ^{1}H n.m.r. (Found: C, 60·8; H, 5·0; N, 21·8%).

8-(4′-Chlorophenyl)-3-methylpteridine-2,4(3H,8H)-dione (3a)

A glyoxal solution (1 g of 30% glyoxal solution, 5·6 mmol) in methanol (10 ml) was added to a suspension of 5-amino-6-(4′-chloroanilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (2e) (1·5 g, 5·6 mmol) in water (50 ml); after brief stirring at room temperature the mixture was refluxed for 30 min. After crystallization was complete the solid was filtered off, washed with ether, and recrystallized from glacial acetic acid to give yellow crystals of (3*a*) (0·75 g, 46%), dec. 333° (Found: C, 54·1; H, 3·2; N, 19·3. $C_{13}H_9ClN_4O_2$ requires C, 54·1; H, 3·1; N, 19·4%). *m/z* (rel. int.) 289 (9%), 288 M (35), 287 (28), 286 (100), 230 (34), 202 (27). ^{1}H n.m.r. (CD_3SOCD_3) δ 3·21, s, CH_3N; 7·60, d, $J_{2',3'}$ 8·9 Hz, H 2′,6′; 7·72, d, $J_{2',3'}$ 8·9 Hz, H 3′,5′; 8·20, d, $J_{6,7}$ 4 Hz, H 6; 8·41, d, $J_{6,7}$ 4 Hz, H 7.

8-(3′,5′-Dimethylphenyl)-3-methyllpteridine-2,4(3H,8H)-dione (3b)

5-Amino-6-(3′,5′-dimethylanilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (2f) (1·5 g, 5·6 mmol) was treated as for (3a) above. The product was recrystallized from 70% ethanol to give brown orange crystals of (3*b*) (0·25 g, 16%), dec. 280° (Found: C, 64·1; H, 5·0; N, 20·1. $C_{15}H_{14}N_4O_2$ requires C, 63·8; H, 5·0; N, 19·9%). *m/z* (rel. int.) 282 M (53%), 281 (100), 267 (73), 225 (46), 224 (54), 210 (50), 197 (43), 196 (55). ^{1}H n.m.r. (CD_3SOCD_3) δ 2·35, s, (CH_3)$_2$C$_6$H$_3$; 3·21, s, CH_3N; 7·15, s, H 2′,6′; 7·24, s, H 4′; 8·17, d, $J_{6,7}$ 4 Hz, H 6; 8·37, d, $J_{6,7}$ 4 Hz, H 7.

8-(4′-Chlorophenyl)-3,6,7-trimethylpteridine-2,4(3H,8H)-dione (3c)

Biacetyl (0·5 g, 5·6 mmol) in methanol (10 ml) was added to a suspension of 5-amino-6-(4′-chloroanilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (2e) (1·6 g, 5·6 mmol) in water (50 ml) and was refluxed for 1 h. After crystallization was complete the solid was filtered off, washed with water and then ether, and recrystallized from methanol to give yellow crystals of (3*c*) (0·5 g, 28%), dec. 261–263° (Found: C, 56·6; H, 4·1; N, 17·6. $C_{15}H_{13}ClN_4O_2$ requires C, 56·9; H, 4·1; N, 17·7%). *m/z* (rel. int.) 318 (18%), 317 (39), 316 M (59), 315 (100), 258 (18), 231 (23). ^{1}H n.m.r. ($CDCl_3$) δ 2·27, s, 6-CH_3; 2·67, s, 7-CH_3; 3·41, s, CH_3N; 7·19, d, $J_{2',3'}$ 8·9 Hz, H 2′,6′; 7·60, d, $J_{2',3'}$ 8·9 Hz, H 3′,5′.

8-(3′,5′-Dimethylphenyl)-3,6,7-trimethylpteridine-2,4(3H,8H)-dione (3d)

5-Amino-6-(3′,5′-dimethylanilino)-3-methylpyrimidine-2,4(1*H*,2*H*)-dione (2f) (1·5 g, 5·6 mmol) was treated as for (3c) above. The product was recrystallized from 70% ethanol to give brown orange crystals of (3*d*) (0·3 g, 17%), dec. 280° (Found: C, 65·8; H, 6·0; N, 18·3. $C_{17}H_{18}N_4O_2$ requires C, 65·8; H, 5·9; N, 18·1%). *m/z* (rel. int.) 310 M (88%), 309 (100), 295 (36), 253 (15), 252 (16), 238 (10), 225 (17), 224 (21). ^{1}H n.m.r. ($CDCl_3$) δ 2·26, s, 6-CH_3; 2·39, s, (CH_3)$_2$C$_6$H$_3$; 2·67, s, 7-CH_3; 3·45, s, CH_3N; 6·77, s, H 2′,6′; 7·18, s, H 4′.

8-(4′-Chlorophenyl)-3-methyl-6,7-diphenylpteridine-2,4(3H,8H)-dione (3e)

A solution of benzil (1·3 g, 6 mmol) in methanol (30 ml) was added to 5-amino-6-(4′-chloroanilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (2e) (1·5 g, 6 mmol) in 50% acetic acid (50 ml), and refluxed for 2 h. After crystallization was complete the solid was filtered off, washed with water and then ether, and recrystallized from 70% ethanol to give bright yellow crystals of (3*e*) (1·3 g, 49%), m.p. 313–314° (Found: C, 67·9; H, 3·9; N, 12·8. $C_{25}H_{17}ClN_4O_2$ requires C, 68·1; H, 3·9; N, 12·7%). *m/z* (rel. int.) 441 (16%), 440 M (43), 439 (51), 438 (100), 353 (24). ^{1}H n.m.r. ($CDCl_3$) δ 3·47, s, CH_3N; 7·00–7·30, complex, 14 aromatic H.

8-(3′,5′-Dimethylphenyl)-3-methyl-6,7-diphenylpteridine-2,4(3H,8H)-dione (3f)

5-Amino-6-(3′,5′-dimethylanilino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (2f) (1·6 g, 6 mmol) was treated as for (3e) above. The product was recrystallized from 70% ethanol to give bright yellow crystals of (3*f*) (0·6 g, 24%), m.p. 331–333° (Found: C, 75·0; H, 5·1; N, 12·8. $C_{27}H_{22}N_4O_2$ requires C, 74·6; H, 5·1; N, 12·9%). *m/z* (rel. int.) 434 M (65%), 433 (100), 419 (21), 349 (11), 348 (33). ^{1}H n.m.r. ($CDCl_3$) δ 2·19, s, $(CH_3)_2C_6H_3$; 3·50, s, CH_3N; 6·68–7·30, complex, 13 aromatic H.

Acknowledgments

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