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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C07D 475/14, A61K 31/525	A1	**(11) International Publication Number:** WO 88/ 04658 **(43) International Publication Date:** 30 June 1988 (30.06.88)

(21) International Application Number: PCT/AU87/00428

(22) International Filing Date: 17 December 1987 (17.12.87)

(31) Priority Application Number: PH 9548/86

(32) Priority Date: 17 December 1986 (17.12.86)

(33) Priority Country: AU

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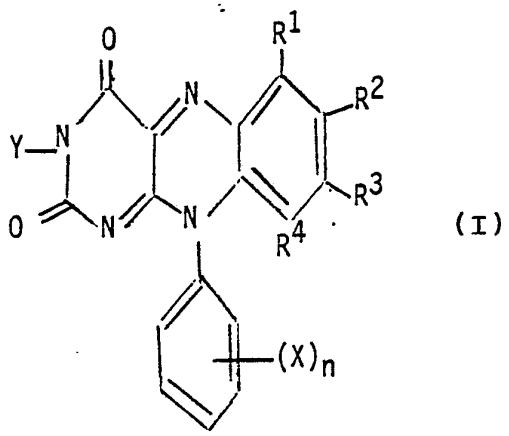
(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: 10-(SUBSTITUTED PHENYL)-3-ALKYLFLAVIN DERIVATIVES HAVING ANTIPROTOZOAL, ANTIPROLIFERATIVE AND ANTIINFLAMMATORY ACTIVITY

(57) Abstract

Compounds of general Formula (I), wherein Y is selected from alkyl, alkenyl, alkynyl, phenylalkyl or substituted or unsubstituted phenyl; n is an integer from 1 to 5; R^1, R^2, R^3, R^4 and X, which may be the same or different, are selected from -Y, -O-Y, -S-Y (wherein Y is as defined above or is unsubstituted or alkyl substituted cycloalkyl and cycloalkenyl), cyclic or non-cyclic amino moieties, halogeno, hydroxyl, thiol or cyano. The 5 N-oxide, dihydro and tetrahydro derivatives are disclosed. Preparation of the above compounds, compositions including the above compounds and methods of using such compositions for antiprotozoal, antiproliferative and/or antiinflammatory treatment of a human or animal are disclosed.



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10-(SUBSTITUTED PHENYL)-3-ALKYLFLAVIN DERIVATIVES HAVING ANTIPROTOZOAL, ANTIPROLIFERATIVE AND ANTIINFLAMMATORY ACTIVITY

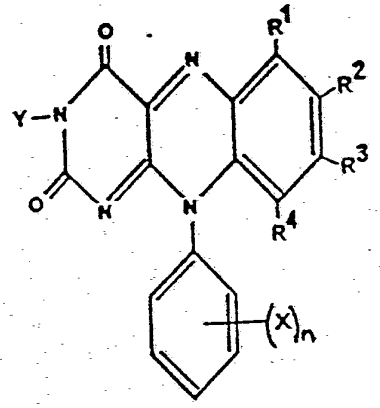
This invention relates to 10-(substituted phenyl)-3-alkylflavin compounds. In particular, the present invention relates to the use of these compounds as selective antiproliferative and antiinflammatory compounds, and as antiprotozoal, more particularly antimalarial, compounds.

The spread of chloroquine and multidrug-resistant strains of Plasmodium falciparum (the primary causative agent of lethal malaria infections in man) has prompted a search for new approaches to antimalarial therapy, and in one approach the antimalarial effects of chronic riboflavin deficiency in animals (1) support an observation made in man(2). Recently, a number of riboflavin antagonists have been shown to have potent antimalarial properties in vitro (3), although depletion of riboflavin in 48 and

96 hour cultures is apparently without effect (4). These same riboflavin antagonists were also shown to have considerable anticoccidial activity in vivo (5). Following this approach, it has recently been suggested (6) that inhibitors of riboflavin metabolism are worth investigating as potential antimalarial drugs.

It has now been found that certain 10-(substituted phenyl)-3-alkylflavin compounds, including some compounds of this group which are novel per se, have significant antimalarial activity in vivo.

According to a first aspect of the present invention, there is provided a group of compounds having antiprotozoal, antiproliferative and/or antiinflammatory activity, said compounds comprising compounds of the general formula I:

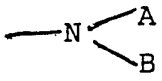


I

wherein Y is selected from the group consisting of alkyl of from 1 to 8 carbon atoms, alkenyl of from 2 to 8 carbon atoms, alkynyl of from 2 to 8 carbon atoms, phenylalkyl of from 7 to 12 carbon atoms, phenyl, alkylphenyl of from 7 to 14 carbon atoms, mono-, di- and trihalogenophenyl, and mono-, di- and tri(trihalogenomethyl)phenyl; n is an integer of from 1 to 5; and R^1, R^2, R^3, R^4 and X, which

may be the same or different, are each selected from the group consisting of:

(a) H, alkyl of from 1 to 8 carbon atoms, alkenyl of from 2 to 8 carbon atoms, alkynyl of from 2 to 8 carbon atoms, unsubstituted and alkyl-substituted cycloalkyl and cycloalkenyl of from 3 to 10 carbon atoms, phenylalkyl of from 7 to 12 carbon atoms, mono-, di- and trihalogenoalkyl of from 1 to 3 carbon atoms, phenyl, alkylphenyl of from 7 to 14 carbon atoms, mono- di- and tri(trihalogenomethyl)phenyl and mono-, di- and trihalogenophenyl;

(b) amino moieties of the formula 

where (i) A and B are each selected from the group consisting of hydrogen, alkyl of from 1 to 8 carbon atoms, alkenyl of from 2 to 8 carbon atoms, alkynyl of from 2 to 8 carbon atoms, unsubstituted and alkylsubstituted cycloalkyl and cycloalkenyl of from 3 to 10 carbon atoms, or

(ii) A and B together with the nitrogen atom to which they are attached represent a heterocyclic moiety selected from the group consisting of aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl, hexahydroazepinyl, heptamethyleneiminyl, morpholinyl, thiomorpholinyl, piperazinyl, 4-alkyl- and 4-arylpiperazinyl, octamethyleneiminyl, each of the said heterocyclic moieties optionally having attached as substituents on carbon atoms

thereof from 1 to 3 alkyl groups of from 1 to 8 carbon atoms;

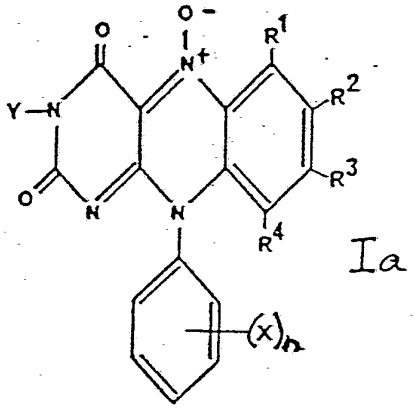
(c) halogeno, including F, Cl, Br, and I;

(d) alkoxy, aryloxy, alkylthio or arylthio moieties of the formulae -O-Z or -S-Z, wherein Z is alkyl of from 1 to 8 carbon atoms, alkenyl of from 2 to 8 carbon atoms, alkynyl of from 2 to 8 carbon atoms unsubstituted and alkylsubstituted cycloalkyl and cycloalkenyl of from 3 to 10 carbon atoms, phenylalkyl of from 7 to 12 carbon atoms, phenyl, alkylphenyl of from 7 to 14 carbons atoms, mono-, di-and tri(trihalogenomethyl)phenyl and mono-, di- and trihalogenophenyl;

(e) hydroxy or thiol; and

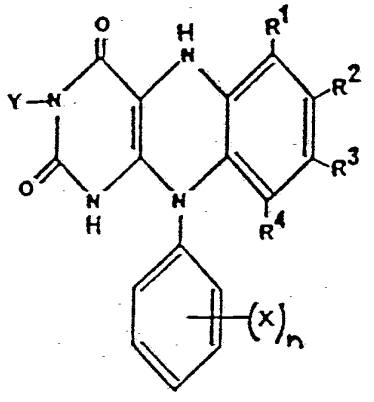
(f) cyano;

or the 5 N-oxide derivatives thereof of the formula Ia



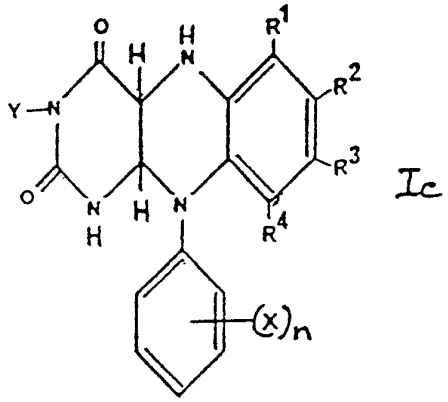
Ia

the dihydro derivatives thereof of the formula Ib



Ib

or the tetrahydro derivatives thereof of the formula Ic



Ic

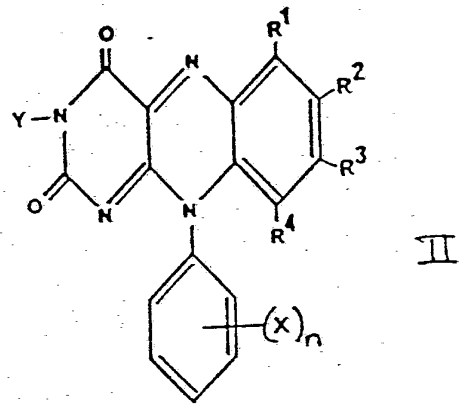
wherein in the formulae Ia, Ib, and Ic, Y, n, R^1, R^2, R^3, R^4 and X are as defined above.

In this aspect, this invention provides a method for the antiprotozoal, antiproliferative and/or antiinflammatory treatment of a human or animal, which comprises the administration to the human or animal of an effective amount of a compound of the general formulae I, Ia, Ib or Ic.

This invention also provides a pharmaceutical or veterinary composition for antiprotozoal, antiproliferative and/or antiinflammatory treatment of a human or animal which comprises a compound of the general formulae I, Ia, Ib or Ic, together with a pharmaceutical or veterinary carrier therefor.

In yet another aspect of the invention there

is provided a class of compounds which are novel per se. These compounds are compounds of the general formula II:



II

wherein Y, n, R^1, R^2, R^3, R^4 and X are as defined above, or the 5N-oxide, dihydro or tetrahydro derivatives thereof, with the proviso that $(X)_n$ is not 4'-chloro, 3'-methyl, 4'-methyl, 2'-ethyl or 2',6'-dimethyl, when Y is methyl and R^1, R^2, R^3 and R^4 are hydrogen.

Particularly preferred compounds of the general formulae I and II are compounds in which Y represents methyl or ethyl.

Also preferred are compounds in which the substituent in the 10 position in general formulae I and II may, for example, be selected from:

2'-chlorophenyl
3'-chlorophenyl
3'-bromophenyl
4'-bromophenyl
3'-fluorophenyl
4'-fluorophenyl
4'-chloro-2'-methylphenyl
2',4'-dichlorophenyl
2',5'-dichlorophenyl
3',5'-dichlorophenyl

4'-chloro-3'-trifluoromethylphenyl
3'-trifluoromethylphenyl
4'-butylphenyl
3'-methoxyphenyl
4'-methoxyphenyl
3'-ethylphenyl
3',5'-dimethylphenyl
4'-diethylaminophenyl
4'-hydroxyphenyl
3'-methylthiophenyl
4'-methylthiophenyl

Preferred compounds are compounds wherein the phenyl group is di-substituted. Particularly preferred compounds are 10-(3',5'-dichlorophenyl) -3-methylflavin and 10-(3',5'-dimethylphenyl) -3-methylflavin.

All of the above compounds are novel and hence fall within both general formula I and general formula II.

Other compounds which fall within general formula I include the compounds:

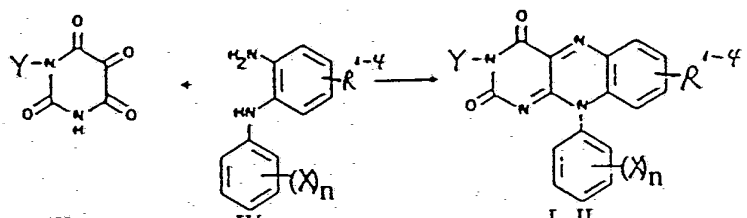
10-(4'-chlorophenyl)-3-methylflavin
10-(3'-methylphenyl)-3-methylflavin
10-(4'-methylphenyl)-3-methylflavin
10-(phenyl)-3-methylflavin
10-(2'-ethylphenyl)-3-methylflavin
10-(2',6'-dimethylphenyl)-3-methylflavin.

The antiprotozoal, antiproliferative and/or antiinflammatory activity of these compounds, was not known prior to the present invention.

Compounds of the general formulae I and II may be made by either of two general methods known in the art for the preparation of 10-phenylflavins.

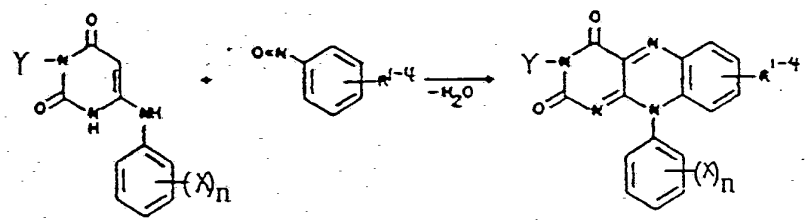
The first of these known methods (7-10)

involves the condensation of an alloxan of the general formula III, with a 2-aminodiphenyl amine of the general formula IV:



III IV I, II.

The second method (11) involves the condensation of a 6-anilinouracil of the general formula V, with a nitrosobenzene of the general formula VI:



V VI I, II.

The 5 N-oxide derivatives (Ia) can be prepared by direct oxidation of the parent compounds (I) with peroxycarboxylic acid reagents (such as m-chloroperoxybenzoic, peroxymaleic or trifluoroperoxyacetic acids) by methods found in the literature (e.g. 12). The di- and tetrahydro derivatives (Ib and Ic) can be prepared by reductions of the parent compounds (I) with catalyst in the presence of hydrogen or by chemical or electrochemical means by methods found in the literature (e.g. 13-17).

Further features of the present invention

will be apparent from the following Examples which illustrate, by way of example only, the preparation and biological activity of compounds of this invention.

EXAMPLE 1

Preparation of 10-(substituted phenyl)-3-methyl or -ethylflavins

A number of 10-(halogenophenyl)-3-methyl and -ethylflavins were prepared by the action of nitrosobenzene on appropriate 3-methyl or 3-ethyl-6-anilinouracils in the presence of acetic anhydride. 10-(4'-chlorophenyl)-3-methylflavin was prepared according to the method of Yoneda et al (11).

Melting points are uncorrected. Analyses were performed 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., U.S.A. or EGA-Chemie, F.R.G.

(a) 6-Arylamino-3-methyluracils

An intimate mixture of 6-chloro-3-methyluracil (1.6g., 10mmol) and the appropriate aniline (30mmol) was heated in an oil bath at 170-175° for 15min. (155-160° for 10min. for compound 1e), cooled briefly and poured into ethanol (35ml) and stirred for 15min. The solid was filtered off, washed with ether (2 x 30ml), recrystallized from acetic acid and dried (Table 1). Analytical samples were prepared from MeOH. 6-Arylamino-4-ethyluracils are prepared by analogous methods.

(b) 10-Halogenophenylflavins

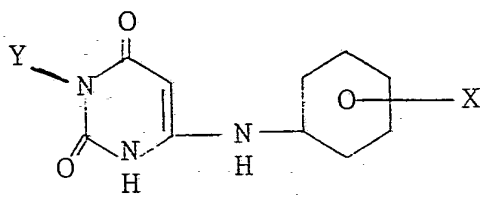
The appropriate 6-anilino-3-methyl or -ethyluracil (10mmol) and nitrosobenzene (3.21g, 30mmol) were refluxed in a mixture of acetic anhydride (16ml) and acetic acid (6ml) for 35min. The volume of the reaction mixture was then reduced by ca 50% under reduced pressure and ethanol (15ml) added. After crystallization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

(c) 10-(2',4'-Dichlorophenyl)-3-methylflavin

6-(2',4'-Dichloroanilino)-3-methyluracil (1.42g, 5mmol) and nitrosobenzene (1.6g, 15mmol) were refluxed in a mixture of acetic anhydride (10ml) and acetic acid (25ml) for 1.5h. The volume was reduced to ca 10ml under reduced pressure and the mixture treated as above to give 10-(2'-,4'-dichlorophenyl)-3-methylflavin. The analytical sample was prepared from acetic acid.

(d) Other 10-(substituted phenyl)-3-methyl and -ethylflavins listed in Table 2 have been prepared by analogous methods.

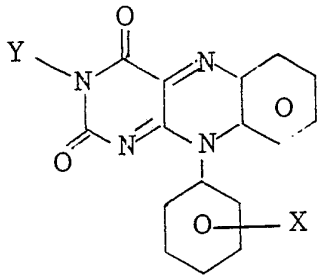
TABLE 1



Y = Me or Et

X	Mp.	% Yield	Formula
4'-Cl	340-342° (lit 257°)	68	$C_{11}H_{10}ClN_3O_2$
2'-Cl	323-324°	88	"
3'-Cl	295-297°	87	"
3'-Br	291-292°	74	$C_{11}H_{10}BrN_3O_2$
4'-Br	335-336°	38	"
3'-F	330-331°	68	$C_{11}H_{10}FN_3O_2$
4'-F	343-344°	79	"
4'-Cl,2'-CH_3	306-307	87	$C_{12}H_{12}ClN_3O_2$
2',4'-Cl_2	370-372°	77	$C_{11}H_9Cl_2N_3O_2$
2',5'-Cl_2	314-316°	81	"
3',5'-Cl_2	328-330°	74	"

TABLE 2



X	Y	Mp.°C	% Yield	Formula
2-Cl	Me	367-368°	53	$C_{17}H_{11}ClN_4O_2$
3-Cl	Me	365-366°	41	"
3-Br	Me	351-353°	47	$C_{17}H_{11}BrN_yO_2$
4-Br	Me	>370°	40	"
3-F	Me	>370°	47	$C_{17}H_{11}FN_4O_2$
4-F	Me	>370°	59	"
4-Cl,2-CH_3	Me	317-320°	20	$C_{18}H_{13}ClN_4O_2$
2,4-Cl_2	Me	347-348°	40	$C_{17}H_{10}Cl_2N_4O2$
2,5-Cl_2	Me	357-359°	43	"
3,5-Cl_2	Me	>370°	40	"
4-Cl,3-CF_3	Me	>370°	44	$C_{18}H_{10}ClF_3N_4O_2$
3-CF_3	Me	344-345°	38	$C_{18}H_{11}F_3N_4O_2$
4-C_4H_9	Me	298-300°	43	$C_{21}H_{20}H_4O_2$
3-OCH_3	Me		48	$C_{18}H_{14}N_4O_3$
4-OCH_3	Me	353-355°	56	"
3-C_2H_5	Me	277-279°	42	$C_{19}H_{16}N_4O_2$
3,5-$(CH_3)_2$	Me	334-335°	42	"
4-$N(CH_2CH_3)_2$	Me	-	-	$C_{21}H_{21}N_5O_2$
4-OH	Me	>370°	69	$C_{17}H_{12}N_4O_3$
4-CF_3	Me	>360°	15	$C_{18}H_{11}F_3N_4O_2$
3-SCH_3	Me	304-305	35	$C_{18}H_{14}N_4O_2S$

4-SCH_3	Me	>360	38	"
2,4-$(CH_3)_2$	Me	333	56	$C_{19}H_{16}N_4O_2$
2-CH_3	Me	>360	50	$C_{18}H_{14}N_4O_2$
2-Et	Me	270-271	23	$C_{19}H_{16}N_4O_2$
4-Et	Me	318-319	51	"
3-SCH_3	Et	264-265	26	$C_{19}H_{16}N_4O_2S$
3-SCH_3	Et	341	43	"
3-CN	Et	349-350	12	$C_{19}H_{13}N_5O_2$
4-CN	Et	>360	32	"
3,5-$(CH_3)_2$	Et	276	36	$C_{20}H_{18}N_4O_2$
3-CF_3	Et	297-300	14	$C_{19}H_{13}F_3N_4O_2$
4-F	Et	>360	33	$C_{18}H_{13}N_4O_2F$
3-F	Et	322	25	$C_{18}H_{13}N_4O_2F$
4-Cl	Et	>360	15	$C_{18}H_{13}N_4O_2Cl$
4-Br	Et	>360	42	$C_{18}H_{13}N_4O_2Br$
3,5-Cl_2	Et	317	21	$C_{18}H_{12}N_4O_2Cl_2$

EXAMPLE 2

Biological Activity

Female CBA/CaH mice, 6-8 weeks old, were used in all *in vivo* experiments. Stock cultures of *P.vinckei vinckei* (strain V52, from F.E.G. Cox) were stored in liquid nitrogen and had been passaged several times in CBA mice before use. All infections were initiated by intraperitoneal injection with 5 x 10^5 parasitised red blood cells and monitored by examiing stained thin smears (Diff-Quik stain set, Australian Hospital Supply, Sydney). Infections became patent on the fourth day and rose exponentially to reach 40-50% parasitemia by day 7, death occurring within a further 2 days.

In vitro studies were performed with parasites of a Papua New Guinea (PNG) strain of *Plasmodium falciparum* (FC27). These were maintained in routine culture using group O erythrocytes cultured in 10% (v/v) O serum in RPMI 1640 (Gibco Ltd.) supplemented with 25mM HEPES, 26mM sodium bicarbonate and 100µg/ml gentamicin (Garamycin,

Flow Ltd.). Flasks were gassed with 5% CO_2, 5% O_2, 90% N_2 and the medium renewed daily. Fresh erythrocytes were added every two to three days.

(a) In vivo drug trials

In order to screen for activity, the drugs were finely powdered, then suspended in propylene glycol and administered to groups of 5, 10 or 20 mice in doses of 25, 50, 75, 100 or 150mg/kg by gavage (50µl). Control animals received propylene glycol alone. A single dose was given when parasitemias reached 30-40% and the efficacy determined by examining stained thin smears at 24 and 48 hours post treatment. Animals were considered to be cured when no parasites were evident 60 days later.

Further experiments were conducted including treatment orally with multiple doses. The drugs were also administered by intraperitoneal and subcutaneous injection as a suspension in olive oil (50µl), control animals receiving olive oil alone.

Using these methods the following tabularized results were obtained and are presented as examples of the efficacy of 10-(substituted phenyl)-3-alkylflavins against plasmodia.

TABLE 3A Percent of cured mice out of 10 treated with a single intraperitoneal dose of 10-(substituted phenyl)-3- methylflavin.

Drug	Dose (mg/kg) 10	15	20	25
3a	60	95	100	90
3b	80	100	100	60
3c	-	60	100	60
3d	100	100	100	100
3e	40	100	100	100
3f	100	90	20	-

TABLE 3B Percent of cured mice out of 10 treated with a single oral dose of 10-(substituted phenyl)-3-methylflavin.

Drug	Dose (mg/kg) 25	50	75	100	150
3a*	45	75	85	85	90
3b	60	100	100	100	100
3c	80	-	-	-	40
3d	-	100	100	100	100
3e	80	80	80	90	100
3f	100	100	80	-	-

*out of 20 treated mice

Drugs: 3a: 10-(4'-chlorophenyl)-3-methylflavin
3b: 10-(4'-bromophenyl)-3-methylflavin
3c: 10-(3',4'-dichlorophenyl)-3-methylflavin
3d: 10-(3',5'-dichlorophenyl)-3-methylflavin
3e: 10-(3',5'-dimethylphenyl)-3-methylflavin
3f: 10-(3'-trifluoromethylphenyl)-3-methylflavin

(b) In vitro drug trials

The drugs were dissolved in dimethyl sulfoxide and incubated overnight at 37°C with group O human serum. Small volumes of this drug-treated serum (1-10µl, containing 3.5 to 35µg/ml) were added to triplicate flat bottom wells of micro-culture trays (Linbro) containing 100µl of a 1% suspension of parasitised erythrocytes (0.5-5% parasitemia, asynchronous parasites) in culture medium without other serum. Normal untreated serum was added to wells containing less than 10% (v/v) serum to bring the final concentration to this amount. Serum amount with the equivalent volume of dimethyl sulfoxide (maximum of 40µl/ml) was added to control wells.

In experiments involving radioactive uptake

of a metabolic label, 20µl of [^{3}H]-hypoxanthine (1/100 dilution of 1 mCi/ml, 2.8 Ci/mmol, Amersham Australia) in RPMI was added to the wells. At the end of culture, the cells were harvested onto filters with a "skatron" cell harvester using water as the washing fluid. The filters were then counted by liquid scintillation. Inhibition of parasite growth was calculated from the level of incorporation of [^{3}H]hypoxanthine in control wells. In some experiments parasite growth was assessed in Giemsa-stained smears made from the contents of culture wells.

The micro-culture trays were incubated for 24 or 48 hours in the usual gas mixture or in one containing a higher oxygen level, namely 5% CO_2 in air (19% O_2).

Using these methods it was demonstrated by way of example that the in vitro growth of P.falciparum was inhibited by 10-(4'-chlorophenyl) -3-methylflavin at a concentration of 4-8µM. Inhibition increased from 53 to 83% at 8µM when the incubation was increased from 24 to 48 hours. The results are shown in Figure 1 which plots antimalarial activity of 10-(4'-chlorophenyl)-3-methylflavin against P.falciparum in 24 hour (◇) and 48 hour (●) cultures as assessed by incorporation of [^{3}H]-hypoxanthine. Points are means of triplicate wells in four separate experiments. At higher oxygen levels (19%) the inhibition was not significantly increased (10%) at 4µM and 0.8µM in 48 hour cultures (3 experiments, data now shown). There was no obvious hemolysis of parasitised cells in drug treated cultures but parasite development was retarded as assessed on Giemsa-stained smears, and a

high percentage of abnormal forms, mostly undeveloped rings or trophozoites, were observed.

(c) Other biological activity

(i) The compounds of this invention have cytostatic and cytoxic activity against a rat mammary adenocarcinoma cell line in culture. The rat mammary adenocarcinoma cells, MAT 13762, were originally from the Mason Research Laboratories, Worcester, MA. and were routinely cultured in RPMI 1640 medium supplemented with 10% heat-inactivated foetal calf serum. These cells were poorly adherent and grew mainly in suspension. By way of example, when cultured in the presence of 10-(4'-chlorophenyl)-3-methylflavin at 4μM concentration, the tumour cells ceased growing after 24 hours as assessed by cytometry. The same compound at 10μM and higher concentrations was cytostatic in 24 hour cultures and obviously cytotoxic in 48 hour and longer cultures as assessed cytometrically.

(ii) The compounds of this invention also inhibit phorbol myristate acetate induced chemiluminescence in both rat and human polymorphonuclear leucocytes in culture and thus have potential antiinflammatory activity.

Rat peritoneal exudate cells were elicited with 12% sodium caseinate and after 16 hours sacrificed and the cells removed. The cells were layered onto percoll gradient d 1.070/1.079/1.080 and spun at 600 x g for 20 minutes. A 99.8% pure

polymorphonuclear (PMN) population was isolated from the bottom of the tube. The cells were washed twice and resuspended at 1 x 10^6 cells per ml in phosphate buffered saline/glucose (5μM). The cell suspension was treated with luminol (113μM) and incubated for 5 minutes.

At this point, the test compound in 2μl of DMSO (test) or 2μl of DMSO alone (control) was added to the cell suspension. After 1 minute of incubation phorbol myristate acetate (10^{-7}M) was added and the resulting chemiluminescence measured. By way of example, 10-(4'-chlorophenyl)-3-methylflavin produced 50% inhibition at 2μM and 74% inhibition at 8μM, 10-(3',5'-dichlorophenyl) -3-methylflavin produced 52% inhibition at 10μM, 10-(3',5'-dimethylphenyl)-3-methylflavin produced 37% inhibition of 10μM and 10-(3'-trifluoromethyl-phenyl)-3-methylflavin produced 40% inhibition at 10μM.

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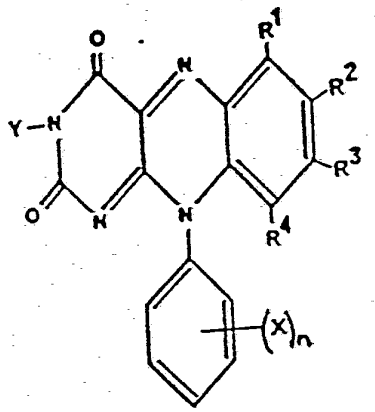
CLAIMS:

1. A method for the antiprotozoal, antiproliferative and/or antiinflammatory treatment of a human or animal, which comprises the administration of an effective amount of a compound of the general formula I:



I

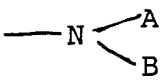
wherein Y is selected from the group consisting of alkyl of from 1 to 8 carbon atoms, alkenyl of from 2 to 8 carbon atoms, alkynyl of from 2 to 8 carbon atoms, phenylalkyl of from 7 to 12 carbon atoms, phenyl, alkylphenyl of from 7 to 14 carbon atoms, mono-, di- and trihalogenophenyl, and mono-, di- and tri(trihalogenomethyl)phenyl;

n is an integer of from 1 to 5; and

R^1, R^2, R^3, R^4 and X, which may be the same or different, are each selected from the group consisting of:

(a) H, alkyl of from 1 to 8 carbon atoms, alkenyl of from 2 to 8 carbon atoms, alkynyl of from 2 to 8 carbon atoms, unsubstituted and alkyl-substituted cycloalkyl and cycloalkenyl of from 3 to 10 carbon atoms, phenylalkyl of from 7 to 12 carbon atoms, mono-, di- and trihalogenoalkyl of from 1 to 3 carbon atoms, phenyl, alkylphenyl of from

7 to 14 carbon atoms, mono- di- and tri(trihalogenomethyl)phenyl and mono-, di- and trihalogenophenyl;

(b) amino moieties of the formula 

where (i) A and B are each selected from the group consisting of hydrogen, alkyl of from 1 to 8 carbon atoms, alkenyl of from 2 to 8 carbon atoms, alkynyl of from 2 to 8 carbon atoms, unsubstituted and alkylsubstituted cycloalkyl and cycloalkenyl of from 3 to 10 carbon atoms, or

(ii) A and B together with the nitrogen atom to which they are attached represent a heterocyclic moiety selected from the group consisting of aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl, hexahydroazepinyl, heptamethyleneiminyl, morpholinyl, thiomorpholinyl, piperazinyl, 4-alkyl- and 4-arylpiperazinyl, octamethyleneiminyl, each of the said heterocyclic moieties optionally having attached as substituents on carbon atoms thereof from 1 to 3 alkyl groups of from 1 to 8 carbon atoms;

(c) halogeno, including F, Cl, Br, and I;

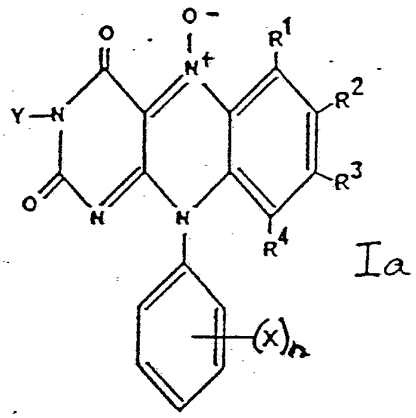
(d) alkoxy, aryloxy, alkylthio or arylthio moieties of the formulae -O-Z or -S-Z, wherein Z is alkyl of from 1 to 8 carbon atoms, alkenyl of from 2 to 8 carbon atoms, alkynyl of from 2 to 8 carbon atoms unsubstituted and alkylsubstituted cycloalkyl and cycloalkenyl of from 3 to 10

carbon atoms, phenylalkyl of from 7 to 12 carbon atoms, phenyl, alkylphenyl of from 7 to 14 carbons atoms, mono-, di-and tri(trihalogenomethyl)phenyl and mono-, di- and trihalogenophenyl;

(e) hydroxy or thiol; and

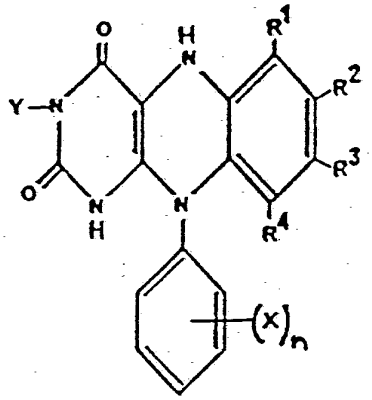
(f) cyano;

or the 5 N-oxide derivatives thereof of the formula Ia



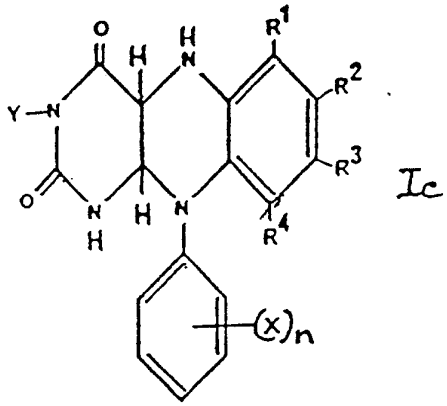
Ia

the dihydro derivatives thereof of the formula Ib



Ib

or the tetrahydro derivatives thereof of the formula Ic



Ic

wherein in the formulae Ia, Ib, and Ic, Y, n, R^1, R^2, R^3, R^4 and X are as defined above.

2. A method according to claim 1, wherein in the compound of the general formulae I, Ia, Ib or Ic, n is 2.

3. A method according to claim 1, wherein in the compound of the general formulae I, Ia, Ib or Ic, y represents methyl or ethyl.

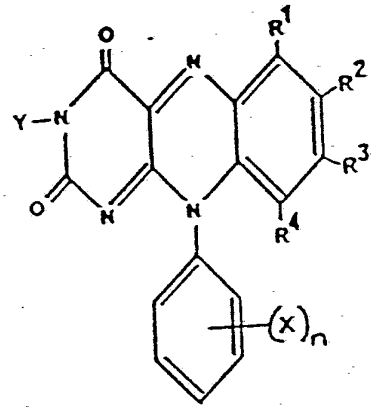
4. A method according to claim 1, which comprises the administration of an effective amount of a compound selected from 10-(3',5'-dichlorophenyl) -3-methylflavin and 10-(3',5'-dimethylphenyl)-3-methylflavin.

5. Use of a compound of the general formulae I, Ia, Ib or Ic as defined in claim 1, for the manufacture of a medicament for the antiprotozoal, antiproliferative and/or antiinflammatory treatment of a human or animal.

6. A pharmaceutical or veterinary composition for antiprotozoal, antiproliferative and/or antiinflammatory treatment of a human or animal which

comprises a compound of the general formulae I, Ia, Ib or Ic, as defined in claim 1, together with a pharmaceutical or veterinary carrier therefor.

7. A compound of the general formula II:



II

wherein Y, n, R^1, R^2, R^3, R^4 and X are as defined in claim 1 or a 5N-oxide, dihydro or tetrahydro derivative thereof, with the proviso that $(X)_n$ is not H, 4'-chloro, 3'-methyl, 4'-methyl, 2'-ethyl or 2',6'-dimethyl, when Y is methyl and R^1, R^2, R^3 and R^4 are hydrogen.

8. A compound according to claim 7 wherein n is 2.

9. A compound according to claim 7, wherein Y represents methyl or ethyl.

10. A compound according to claim 7, selected from 10-(3',5'-dichlorophenyl)-3-methylflavin and 10-93',5'-dimethylphenyl)-3-methylflavin.

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 87/00428

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] C07D 475/14, A61K 31/525

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	C07D 475/14, A61K 31/525

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

AU : IPC as above
Chemical Abstracts

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	AU,B, 69747/74 (478900) (MORTON-NORWICH PRODUCTS, INC.) 4 December 1975 (04.12.75)	(1-10)
X	Biochemistry, Volume II, no.21, published 1972, October 10 (Washington, D.C., U.S.A.) Main et al, 'Kinetics and Mechanism of the Isoalloxazine (Flavine) Dehydrogenation of Dimethyl Dihydrophthalates', see pages 3991-4000, especially compound 11	(7,9)
X	Chemical Abstracts, Volume 104, no.21, issued 1986, May 26 (Columbus, Ohio, U.S.A.) Shinkai et al, 'Synthesis and optical resolution of a new flavin with axial chirality and redox-dependent racemization', see page 614, column 1, abstract no. 186219x	(7,9)
X	Chemical Abstracts, Volume 102, no.25, issued 1985, June 24 (Columbus, Ohio, U.S.A.) Sako et al, 'A new and versatile synthesis of isoalloxazines', see page 590, column 1, abstract no. 220830k	(7,9)

(continued)

* Special categories of cited documents: [14]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 February 1988 (29.02.88)	(29-03-88) 29 MARCH 1988
International Searching Authority	**Signature of Authorized Officer**
Australian Patent Office	C.A. BRICK

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X.	Chemical Abstracts, Volume 102, no.15, issued 1985, April 15 (Columbus, Ohio, U.S.A.) Yano et al, 'Substituent and steric effects of flavin models in the reactions of N-benzyl-1,4-dihydronicotinamide, butane-1,4-dithiol, phenylhydrazine, and nitroethane', see abstract no. 131365v	(7,9)
X	Chemical Abstracts, Volume 92, no.19, issued 1980, May 12 (Columbus, Ohio, U.S.A.) Yoneda et al, 'A new synthesis of 10-arylisoalloxazines(10-arylflavins)', see page 600, column 2, abstract no. 163936y	(7,9)

(continued)

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 1-4 because they relate to subject matter not required to be searched by this Authority, namely:

Methods of treatment of the human or animal body by therapy.

2. [ ] Claim numbers ..... because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

3. [ ] Claim numbers..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Journal of Heterocyclic Chemistry, Volume 16, issued 1979, November, (Tampa, Florida, U.S.A.) Yoneda et al, 'A new synthesis of 10-aryliso-alloxazines (10-arylflavins)', see pages1365-1367, especially compounds 11c-f	(7,9)
X	American Chemical Society Journal, Volume 99, no.22, issued 1977, October 26 (Los Angeles, California, U.S.A.) Chan et al, 'Reaction of Nitroxides with 1,5-Dihydroflavins and N^3,5-Dimethyl-1,5-Dihydrolumiflavin', see pages 7287-7291, especially compound V	(7,9)
X	American Chemical Society Journal, Volume 93, no.26, issued 1971, December 12 (Los Angeles, California, U.S.A.) Bruice et al, 'Preequilibrium Complex Formation and Nucleophilic Addition (and its position) as factors in Flavin-Catalysed Oxidations', see pages 7327-7328, especially compound 11	(7,9)
X	Chemical Abstracts, Volume 88, no.13, issued 1978, March 27 (Columbus, Ohio, U.S.A.) Yoneda et al, 'A new, facile synthesis of 10-arylisoalloxazines', see abstract no. 89627x, especially compound I	(7,9)
X	Chemical Abstracts, Volume 80, no.1, issued 1974, January 7 (Columbus, Ohio, U.S.A.) Elliot D., 'Properties of peroxide-reactivated phenylmethane-sulfonylchymotrypsin. Reactions of anions with the flavin model compound 3-benzyl-10-phenyliso-alloxazine', see page 1106, column 1, abstract no. 1096z	(7)
X	Chemical Abstracts, Volume 88, no. 15, issued 1977, October 5 (Columbus, Ohio, U.S.A.) Yoneda et al, 'One step Synthesis of 8-Chloroflavins by the Cyclization of 5-Nitro-6-(N-substituted-anilino) uracils with the Vilsmeier Reagent. Vilsmeier Reagent as a Reducing Agent', see abstract no. 105268p, especially compound II	(7)
X	Journal of the Chemical Society: Chemical Communications, Volume 14, issued 1972, July 19 (London, England) Main et al, 'Isoalloxazine (Flavin) Dehydrogenation of Dimethyl trans-1,2-Dihydrophthalate', see pages 847-848, especially compound V	(7,9)
X	Chemische Berichte, Volume 107, No.5, issued 1974, May 13, (Weinheim, West Germany) Knappe R., 'Photochemie des 10-Phenylisoalloxazins : Intramolekulare Singulette-und intermolekulare Triplett-Reaktionen', see pages 1614-1636, especially compound 3	(7,8)

(continued)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	American Chemical Society Journal, Volume 99, No.22, issued 1977 (Los Angeles, California, U.S.A.) Kemal et al, 'Reaction of O_2 with Dihydroflavins.1. N^3-5-Dimethyl-1,5-Dihydrolumiflavin and 1,5-Dihydro-isoalloxazines', see pages 7272-7288	
A	Journal of the Chemical Society: Chemical Communications, Volume 24, issued 1975 (London, England) Sakuma et al, 'New Synthesis of Flavins', see pages 977-978	
A	Journal of the American Chemical Society, Volume 98, no.3, issued 1976 (Los Angeles, California, U.S.A.) Yoneda et al, 'Synthesis of Isoalloxazines and Iso-alloxazine 5-oxides. A new synthesis of Riboflavin', see pages 830-835	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 87/00428

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
AU 478900	BE 820110	CA 1031337	DE 2444394
	ES 430220	FR 2243697	GB 1420727
	JP 50053397	NL 7408066	SE 7410164
	US 3920650		

END OF ANNEX