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ANTIMALARIAL ACTIVITY OF HYDROXY-SUBSTITUTED NAPHTHALENE COMPOUNDS

BY

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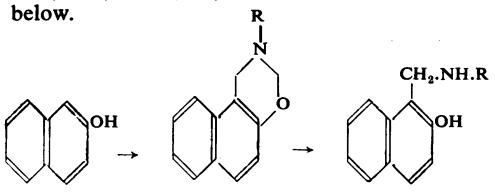
A new series of mono- and di-hydroxy substituted naphthalene compounds was synthesized and found to possess antimalarial activity against *P. gallinaceum* infection of young chicks. A representative compound with a high degree of activity was chosen for extensive testing against other malarial species and for pharmacological investigation. The formula of this compound, number 377C54, was 1:6-dihydroxy-2:5-bis(*cyclo*hexylaminomethyl)naphthalene dihydrochloride. Effective doses against *P. gallinaceum* in chicks, *P. berghei* in mice and *P. cathemerium* in canaries were 2.3, 4.0, and about 6 mg./kg. respectively. Compound 377C54 acted rapidly against the parasitaemia of *P. gallinaceum* in chicks and *P. knowlesi* in a rhesus monkey. Parasiticidal activity remained in the blood of chicks for a long time after a single oral dose. The drug can be estimated by the production of colour on coupling with diazotized *p*-nitroaniline. Drug concentrations in blood from chicks and humans rose rapidly after oral administration. In tissues from chicks, particularly liver and lung, the drug persisted for a long period. An unsuccessful attempt was made to induce resistance to 377C54 in a strain of *P. gallinaceum*. Extensive pharmacological investigation showed that 377C54 possessed no special pharmacological properties.

Several derivatives of 1- and 2-naphthol were tested for antimalarial activity in the American wartime screening programme (Wiselogle, 1946). One member of the series, which showed activity against laboratory malaria infections, SN 6520 [2-(dimethylaminomethyl)-1-naphthol], was given clinical trial against blood-induced *Plasmodium vivax* infections. It was found to be inactive at doses of 2 g. daily. No tests were made for either prophylactic or curative activity.

The experiments recorded here are the result of the observation that 1-*cyclo*hexylaminomethyl-2-naphthol (371C52) showed antimalarial activity against blood-induced *P. gallinaceum* infection in young chicks.

METHODS

The compounds were made by the method described by Burke, Kolbezen, and Stephens (1952). The corresponding naphthols were reacted with formaldehyde and a primary amine to give an oxazine which was then hydrolysed by hydrochloric acid in propanol, as below.



Screening and assaying of new compounds were carried out using *P. gallinaceum* infections of 5- or 12-day-old chicks. The methods used were those previously described by Falco, Goodwin, Hitchings, Rollo, and Russell (1951). Where compounds were also tested against *P. berghei* in mice the technique of the same authors was used. Where only a screening test was carried out the compound was awarded a "score" according to the following key:

0=No activity at 100 mg./kg.
1=Slight activity at 100 mg./kg.
2=Active at doses between 100 and 10 mg./kg.

A dose was considered to be "active" when the mean % parasitaemia of the treated group of animals was lowered to 1% or less of the mean % parasitaemia of untreated controls. When an assay was carried out, the median effective dose (ED50) was determined. This is defined as the dose which reduces parasitaemia to 50% of the mean parasitaemia of untreated controls. A 3- or 4-dose assay was used as described by Rollo (1952).

Compound 377C54 was also tested against blood-induced infections of *P. cathemerium* in canaries and *P. knowlesi* (nuri strain) in a rhesus monkey. Canaries were inoculated intravenously with approximately 25×10^6 parasitized red blood cells, and were given a course of treatment lasting four days as in the tests using *P. gallinaceum*. The monkey was infected by the intraperitoneal injection of 3 ml. of infected citrated blood and was given single oral doses to assess

the effect of the drug on a moderately high parasitaemia. The drug was given orally in solution. The blood was examined by prolonged search of thin films. Thick films were not made. Single oral doses were also given to chicks infected with *P. gallinaceum* to determine the speed of action of the compound on an established infection. The appearance of the parasites at various times after dosing was assessed from an examination of stained blood films.

In some experiments single doses of drug were given to uninfected chicks and the persistence of the substance in the blood was assessed by challenge at intervals by intravenous inoculation of parasitized red blood cells. The details of this test are as follows: (i) The dose of compound 377C54 used was 200, 50, or 12.5 mg./kg. (ii) Young chicks were infected by an inoculum of *P. gallinaceum* containing 5×10^7, 5×10^6, or 5×10^5 parasitized red cells. (iii) The time interval between dosing and inoculation was 24, 48, or 96 hr. The chicks were examined daily for 11 days after inoculation and the mean number of days free from parasites for each group was estimated using the formula,

$$(1/N_0)(\tfrac{1}{2}N_0 + N_1 + N_2 + \ldots\ldots N_{n-1} + \tfrac{1}{2}N_n)$$

where N_n = number of chicks free from parasites on day n and n = length of experiment in days.

In an attempt to induce resistance to the compound, a substrain from the normal *P. gallinaceum* strain was treated with subcurative amounts over a period of 33 weeks. The strain was passaged many times during the period. Normal assays were carried out during and at the end of the time, to compare the responses of the treated strain with those of the untreated parent strain. Estimations were made of the concentration in the blood and tissues of chicks and in the blood of human volunteers after a single oral dose of a selected member of the series. Before extraction with redistilled ether, the blood was laked in ten times its own volume with 1% alcohol in distilled water; tissues were homogenized. The extraction and all subsequent operations were conducted by the light of a red photographic safelight. The drug was extracted into ether after the *p*H of the sample had been adjusted to 8 with phosphate buffer. The drug was then taken into 5 ml. N/10-HCl and a colour developed with diazotized *p*-nitroaniline. The intensity of the colour was immediately estimated by reading at 465 mμ in a Unicam Spectrophotometer (Model SP 350).

Results

Multiple Dose Experiments

Activity of Some 1-Hydroxynaphthalene Derivatives.—In the examples tested, compounds with a substituted amino methyl group in the 2-position had moderately high antimalarial activity, such as the *cyclo*hexylamino- (ED50 40 mg./kg.) and *n*-butylamino- (score 2) compounds. However, if the aliphatic chain was considerably lengthened, as in octylamino-, activity was lost.

Methoxy-substitution in position 5 had little effect on the activity of the 2-*cyclo*hexylaminocompound, but in position 4 reduced activity. A chlorine atom in the 4-position did not affect activity.

Substances with heterocyclic basic groups in position 2, such as the pyrrolidinomethyl- and piperidinomethyl- compounds, also showed activity (score 2). The non-basic pyrrolidide, which is insoluble, was inactive.

Activity of 2-Hydroxynaphthalene Derivatives.—Compounds with a substituted aminomethyl group in position 1 were active provided the substituent was sufficiently large. The unsubstituted and the methyl- and *iso*propyl- substituted compounds were inactive. Of the compounds tested, optimum activity was found in the aliphatic series with *iso*pentyl (score 2). *cyclo*Pentyl, *cyclo*hexyl, and *cyclo*heptyl derivatives had about the same activity (ED50 70, 98, and 83 mg./kg. respectively). It is interesting that the only tertiary amino compound tested, 1-di-*n*-propylaminomethyl-2-hydroxynaphthalene, was inactive.

Substitution of the hydroxy group in the 1-*cyclo*hexylaminomethyl compound by methoxy resulted in a considerable increase in activity (ED50 58 mg./kg.). Increase of activity was also observed when the unsubstituted ring of the naphthalene nucleus was reduced (score 2). Substitution with methyl, methoxy, or hydroxymethyl groups in position 3 of the naphthalene nucleus led to compounds of lower activity.

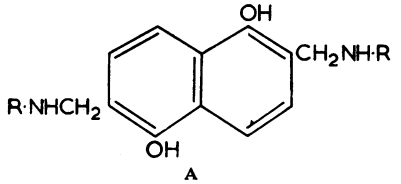
In the 1-*cyclo*hexylaminomethyl series several compounds were prepared with substituents in the *cyclo*hexyl ring. The effect of a methyl group depended on its position: 2-methyl (score 1) gave lower activity than either 3-methyl or 4-methyl (both score 2). Chlorine in position 2 reduced, whereas hydroxyl increased, activity.

Derivatives of three dihydroxynaphthalenes have been investigated (Table I). Owing to instability only one example (382C54) of the 1:5-dihydroxy compounds was prepared. This was less active than the 1:6 and 2:6 isomerides.

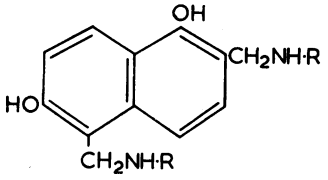
Among the members of the 1:6-dihydroxy series were found the most active of all the compounds tested. Activity depended on the length of the alkyl substituent of the amino group, the *n*-propyl and the *n*-butyl substituted compounds being the most active of those tested. Lengthening of the chain to 8 carbon atoms (356C55) resulted in a marked decrease in activity. *cyclo*Pentyl, *cyclo*hexyl-, and *cyclo*heptyl- substituted compounds were highly active.

TABLE I

THE ANTIMALARIAL ACTIVITY OF DIHYDROXY-BIS(R. AMINOMETHYL)NAPHTHALENES



A



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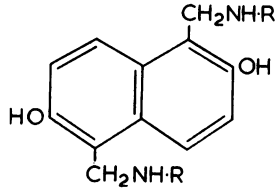


C

The asterisk denotes the mean of several determinations.

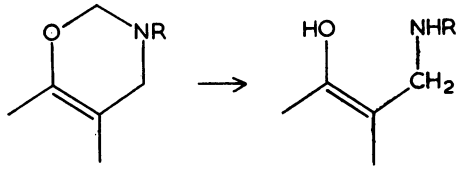
Reference No.	R	Score of Activity or ED50's in Parentheses (mg./kg.)
	Group A 1 : 5-*di*(*OH*)-2 : 6-*bis*(*R.NH.CH*$_2$)	
382C54	*cyclo*Hexyl	(8·1)
	Group B 1 : 6-*di*(*OH*)-2 : 5-*bis*(*R.NH.CH*$_2$)	
672C55	Ethyl	(6·1)
608C55	*n*-Propyl	(1·4)
325C55	*n*-Butyl	(1·5)
356C55	*n*-Octyl	(35·5)
322C55	*cyclo*Pentyl	(1·8)
377C54	*cyclo*Hexyl	(2·3)*
372C55	*cyclo*Heptyl	(2·7)
	Group C 2 : 6-*di*(*OH*)-1 : 5-*bis*(*R.NH.CH*$_2$)	
66C56	Methyl	2
115C56	Ethyl	(10)
97C56	*n*-Propyl	(4·6)
91C56	*iso*-Propyl	(3·9)
26C56	*n*-Butyl	(3·2)
67C56	*n*-Pentyl	(3·4)
322C56	*n*-Hexyl	(8·9)
323C56	*n*-Heptyl	(89)
216C54	*cyclo*Hexyl	(4·3)

Members of the 2:6-dihydroxy series were less active than the corresponding compounds in the 1:6-dihydroxy series. The *n*-butyl compound was the most active.

Since all the aminomethylnaphthols were prepared from the corresponding oxazines, it was thought of interest to examine the activity of some of the oxazines themselves. Assays were carried out with two of the oxazines and the corresponding naphthalenes and the following results obtained:

	ED50 (mg./kg.)
1-hydroxy-2-*cyclo*hexylaminomethylnaphthalene	40
3-*cyclo*hexyl-3 : 4-dihydro-2H-naphth(2 : 1,e)-*m*-oxazine	51
1 : 5-di(hydroxy)-2 : 6-bis(*cyclo*hexylaminomethyl) naphthalene	8.1
2 : 8-*cyclo*hexyl-1 : 2 : 3 : 4 : 7 : 8 : 9 : 10-octahydro-2 : 8-diaza-4 : 10-dioxachrysene	7.8

It is obvious that the ED50's of the related pairs were of the same order—indeed those of the latter pair, allowing for experimental error, can be said to be the same. The evidence, therefore, points to a similarity of action in the paired compounds, and it seems probable that the oxazines break down *in vivo* to the corresponding active compound thus:



Because of its marked antimalarial action, compound 377C54 [1 : 6-dihydroxy-2 : 5-bis(*cyclo*hexylaminomethyl)naphthalene dihydrochloride] was selected to undergo extensive testing against laboratory malarial infections and pharmacological investigation.

The results in Table II show that the high activity shown against *P. gallinaceum* in chicks is also shown against *P. berghei* in mice and *P. cathemerium* in canaries.

TABLE II

ASSAY OF COMPOUND 377C54 AGAINST THREE SPECIES OF MALARIAL PARASITE

Species	Drug	Dose (mg./kg.)	Mean % Parasitaemia	ED50 (mg./kg.)
P. gallinaceum ..	377C54	5 3·54 2·5	<1 21 76	3·3
	Untreated controls	—	79	—
P. berghei ..	377C54	6 3 1·5	<1 29 55	4·0
	Untreated controls	—	33	—
P. cathemerium	377C54	10 2·5	0 19	*ca.* 6
	Untreated controls	—	18	—

Single Dose Experiments

Effect of a Single Oral Dose on High Parasitaemia

P. gallinaceum *in Young Chicks.*—Single oral doses of 10 mg. base/kg. of chloroquine and of 377C54 affected the parasites of an established infection of *P. gallinaceum* in chicks after 4 hr., and after 8 hr. nearly all the parasites in stained smears showed degenerative changes. These changes appeared in trophozoites; young merozoites and mature schizonts seemed to be unaffected. Doses of 2.5 mg./kg. of either compound had no effect upon the morphology of the parasites. The new compound had a speed of action equal to that of chloroquine.

P. knowlesi *in a Rhesus Monkey.*—Three days after inoculation, examination of the monkey's blood showed the presence of numerous parasites. The first dose was then administered; subsequent doses were given whenever parasites again appeared in the blood in any number.

The rapidity of action of the drug which became evident from the experiments with *P. gallinaceum* was also noted with *P. knowlesi.* After 50 mg./kg. all parasites had disappeared from the blood within 17 hr. and remained absent for 10 days. With 20 and 10 mg./kg., parasites disappeared within 24 hr. and remained absent for 7 and 3 to 5 days respectively. When 5 mg./kg. was given, a single parasite was seen 24 hr. later after prolonged search. The blood was free from parasites for 2 days.

It seems unlikely that immunity to the infection influenced the later results, because the length of time free from parasitaemia was roughly proportional to the dose used, and because when immunity was established the animal was free from parasites for many weeks.

This strain is known to be extremely virulent in rhesus monkeys: if left untreated, the parasites rapidly multiply and the monkey dies in 7 to 14 days.

Persistence of Parasiticidal Activity in Blood after a Single Oral Dose

Parasiticidal activity remained in the blood for a considerable length of time after a single oral dose, as is shown in Table III. Two explanations can be put forward. Either the drug, or a possible metabolite, remained in the blood perhaps bound to plasma protein, or, as is well known to be the case with chloroquine, the drug was first taken up by the tissues and then slowly liberated.

In order to obtain more evidence on these points chemical estimations of blood and tissue concentrations were carried out.

TABLE III

MEAN NUMBER OF DAYS FREE FROM INFECTION OF *P. GALLINACEUM* AFTER A SINGLE DOSE OF 377C54 RELATED TO THE INTERVAL BETWEEN DOSE AND INOCULATION

Each figure in the Table represents the mean number of days free from infection, and, where this figure is greater than that for the corresponding untreated control group, it appears in bold type.

Interval Between Dose and Inoculation (hr.)	Dose 377C54 (mg./kg.)	No. of Days Free from Infection		
		Inoculum (Approx. Number Parasitized Red Cells)		
		5×10^7	5×10^5	5×10^3
24	200	**9·5**	**10·8**	**11**
	50	**9**	**9**	**>11**
	12·5	<4	**5·8**	**>11**
	—	<4	<4	7·3
48	200	**8·6**	**>10**	**>11**
	50	**7·3**	**9·2**	**>11**
	12·5	<4	4	8·3
	—	<4	<5	8·5
96	200	**6·5**	**8·8**	**>11**
	50	<4	<5	8
	12·5	<4	<5	8·8
	—	<4	<5	>9

Estimation of 377C54 in Blood and Tissues

Blood and Tissues from Chicks given Single Oral Doses of 377C54

Chicks, given 100 mg./kg. of 377C54, were killed at various times after the dose. Table IV shows a series of results obtained when the drug was extracted from blood and liver.

TABLE IV

CONCENTRATION OF 377C54 AFTER SINGLE ORAL DOSE OF 100 MG./KG. IN THE CHICK

Hr. after Dose	Blood Concentration (mg.%)	Liver Concentration (mg./Liver)
¼	0·10	0·01
½	0·16	0·03
1	0·23	0·12
2	0·27	0·18
4	0·07	0·30
7	0·15	0·41

Estimations of drug from tissues taken from a chick weighing 65 g. which had been dosed with 10 mg./kg. of 377C54 24 hr. previously showed that no drug could be recovered from blood, brain, heart, kidney, muscle, or spleen, but that liver and lung contained totals of 40 and 2.0 μg. respectively.

A further batch of chicks was dosed with 10 mg./kg. These were killed at 3, 7, and 11 days afterwards and the concentration of 377C54 in liver and lungs estimated. At the end of these periods the lungs contained 4, 7, and 5 μg. of the drug; at the end of 3 days the liver contained 5 μg., but none could be recovered after 7 days.

The concentration of drug in the blood reached a peak in about 2 hr. and thereafter slowly decreased. The maximal concentration of drug in the liver was attained more slowly, and 3 days after the comparatively small dose of 10 mg./kg. there was still an appreciable amount of drug present. Measurable quantities of drug were still present in lung 11 days after a single oral dose.

Blood and Urine from Human Volunteers

A dose of 600 mg. of 377C54 was taken by mouth by each volunteer. From the results given in Table V it may be concluded that the drug

TABLE V

CONCENTRATION OF 377C54 IN HUMAN BLOOD AND URINE AFTER SINGLE ORAL DOSE OF 600 MG.

Individual	Blood		Urine	
	Interval (hr.)	Concentration 377C54 (μg.%)	Interval (hr.)	Total 377C54 in Sample (μg.)
J. H. ..			2½	Nil
	1	37·5	3	28·4
	2	72·5	5½	48·8
	4	66·6	7	41·3
H. R. ..	1	75·0	2½	Nil
	2	58·0	6	41·5
	4	30·0	8½	5·4
	8	Nil		

rapidly attained a peak of concentration in the blood, but at the same time little was excreted unchanged in the urine. It may be that the drug is metabolized and excreted in a form that cannot be determined by the methods used here. The evidence from estimations of drug in chick tissues suggests that it is taken up and retained, particularly in liver and lung.

Attempt to Induce Drug Resistance to Compound 377C54 in P. gallinaceum

In none of the experiments described under "Methods" was the ED50 for 377C54 against the treated substrain appreciably different from that obtained against the normal strain. There was therefore no development of resistance.

Pharmacological Investigations

The LD50 of 377C54 given intravenously in mice was approximately 35 mg./kg. Marked respiratory distress was observed after 15 mg./kg. In a cat under chloralose anaesthesia, 3 mg./kg. had a mild hypotensive effect and 10 mg./kg. caused cardiac failure with a precipitous fall in blood pressure practically to zero and respiratory arrest. During artificial respiration, the blood pressure returned gradually to near the pre-injection value within 15 min. The hypotensive effect of 377C54 was uninfluenced by ganglion blocking agents or by atropine.

The toxic oral dose of 377C54 in cats was about 400 to 600 mg./kg. Five of ten cats survived such amounts and two of them became blind. In these, ophthalmoscopic examination revealed marked constriction of retinal vessels.

Acute experiments showed the drug to have no important effect on the plain muscle of the ileum or uterus *in vitro*, but concentrations of 10^{-6} to 3×10^{-6} caused some inhibition of the peristaltic reflex. No specific antagonism of acetylcholine or histamine was found in guinea-pig ileum or cat blood pressure experiments, and, except after doses causing severe hypotension (10 mg./kg.), there was no interference with the blood pressure responses to vagal stimulation or $N^1:N^1$-dimethyl-N^2-phenylpiperazinium iodide, or with the response of the nictitating membrane to preganglionic nerve stimulation in an anaesthetized cat. A further indication of lack of ganglion blocking or anti-parasympathetic effects was the absence of a mydriatic effect with 25 mg./kg. 377C54 i.p. in mice. No antagonism of the hypertension caused by adrenaline or (−)-noradrenaline was observed on the cat blood pressure. A concentration of 10^{-6} did not antagonize spasm of the isolated rat uterus preparation elicited by 5-hydroxytryptamine. The duration of pentobarbitone sodium anaesthesia in mice was unaffected by 25 mg./kg., i.v., and the analgesic action was at most slight with 50 mg./kg., s.c., in rats.

The pharmacological investigations were carried out by Dr. Green, Dr. Udall, and Dr. White at the Wellcome Research Laboratories, Beckenham. The authors are indebted to Miss C. Reynolds for her assistance in the laboratory.

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