Antimalarials. Unsaturation in Chloroquine Side Chain and Antimalarial Activity

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Six chloroquine derivatives with unsaturation in the diamine side chain in the form of acetylenic and *cis* and *trans* ethylenic bonds have been prepared and their antimalarial activity has been compared with that of chloroquine against *Plasmodium berghei* in mice. Some of these were found to be more potent and less toxic than chloroquine.

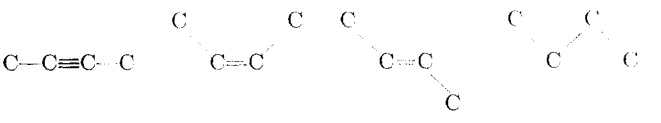
The present study was taken up, as a part of our program on the syntheses of new antimalarial compounds, in order to explore the biological response to a triple bond and a double bond introduced into the diamino side chain of chloroquine (I). The other variation studied along with unsaturation in the diamino side chain is the presence or absence of the 1-methyl group. It becomes evident from the projection models of these variations, that we have several different structures at our disposal, every one of which should be of interest with regard to how the diamino side chain should fit itself into the receptor site of the parasite. There are several other unsaturated structures possible which should be of further interest from this point of view but the structures we selected were the chemically feasible ones and were not likely to bring in any additional chemical influences in the chloroquine molecule, other than the usual chemical reactivity of the carbon–carbon triple and double bonds.

The acetylenic diamines (VI, R = H, CH_3) were obtained according to the method of Dahlbom, *et al.*[1] (Scheme I). The *cis* reduction of the acetylenic amines was carried out with Lindlar catalyst[2,3] according to the procedure described by Ettlinger and Hodgkin.[4] The *cis* reduction of the acetylenic bond was also possible in compound **4** to give **5** in 72.0% yield (Table I).

It has been suggested by Hahn and coworkers[5] that the ring system of chloroquine and quinacrine,[6] in their mode of action, are intercalated into DNA. The side chain apparently falls outside the contour of the DNA base pairs and interacts ionically with phosphate groups of the complementary strands of DNA across the minor groove of the double helix. It has also been stated by these workers that the "distance across the minor groove of DNA from phosphoric acid to phosphoric acid in complementary strands is 10.5 Å." Taking into consideration the ionic radii of the amino group in the side chain of chloroquine, the best interval between the centers of the two N atoms to fit this diamine across the minor groove by electrostatic attraction would be 7.5 Å, *i.e.*, the interval actually existing in the 1,4-diaminopentane chain of chloroquine. This argument is further supported by the fact that chloroquine analogs with three and five C atoms between the two N atoms are less active.

Considering the question of additive bond distances between the two N atoms in the side chain, we find that it varies from 7.56 Å in chloroquine and 7.35 Å in the ethylenic compounds **5** and **6** to 7.22 Å in the acetylenic **4**. The difference between the longest, chloroquine, and the shortest, the acetylenic compound, is only 0.34 Å. In view of the fact that the biological activities of these compounds vary widely, this small difference in the additive bond distance may not be sufficiently significant to account for the considerable differences in activity. Thus, our attention is drawn more toward the spatial differences in the side-chain amines of these molecules. These differences, as engendered by the *cis* and *trans* ethylenic bond, the acetylenic bond, and the absence or presence of the 1-methyl group, do vary considerably. Limiting our argument only to one category of compounds, *i.e.*, the ones containing 1-methyl, we have the saturated side chain of chloroquine, *cis* and *trans* ethylenic bonds, and the acetylenic bond. All four show characteristic spatial arrangements of atoms which are sufficiently different to account for the differences in activity of these compounds. Therefore, it may be suggested that, if in the mode of action of chloroquine the side chain adjusts itself between the phosphate groups of the complementary strands of DNA across the minor groove, then more important than additive bond distances between N atoms is the manner in which this adjustment takes place: *the actual distance between N atoms in the adjustments of various configurations will vary more widely than the additive bond distance.* This statement is, of course, based on the tentative assumption that the unsaturated centers remain intact.

The four carbon atoms between the two N atoms in **4**–**6** and chloroquine are in approximate arrangements as shown below. Chloroquine, in which rotation



around C–C bonds is more labile, can assume the "approximate" arrangement of *trans* **6** and *cis* **5**. Thus the *trans* compound is closer to chloroquine in toxicity in mice than the *cis* compound while both show greater curative activity than chloroquine, particularly *cis* **5**. From this, it might be suggested that chloroquine side chain is adjusted between the two phosphoric acid centers largely in the *trans* configuration and to a small

(1) R. Dahlbom, B. Karlen, R. George, and D. J. Jenden, *J. Med. Chem.*, **9**, 843 (1966).

(2) H. Lindlar, *Helv. Chim. Acta*, **35**, 446 (1952).

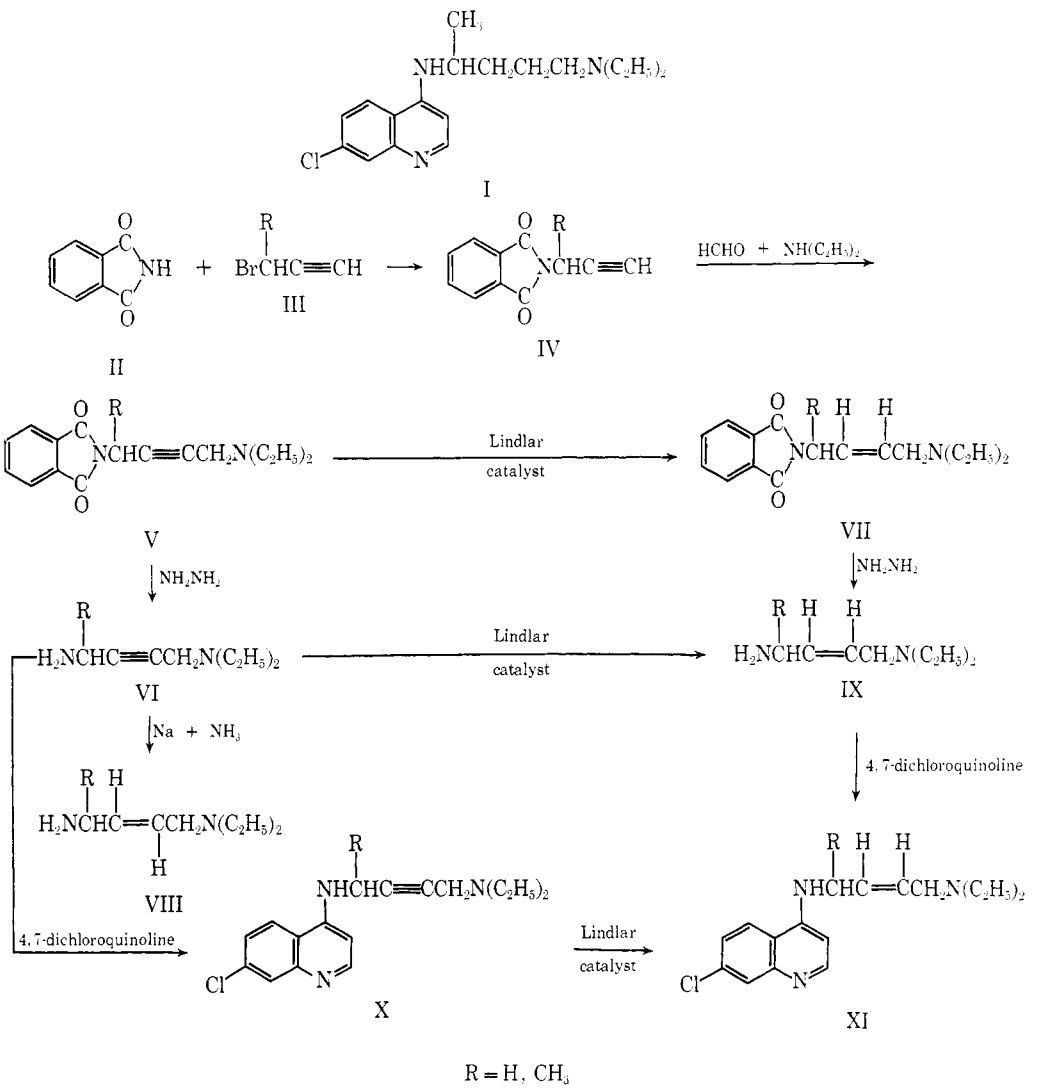
(3) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," John Wiley and Sons, Inc., New York, N. Y., 1967, p 566.

(4) M. G. Ettlinger and J. E. Hodgkin, *J. Amer. Chem. Soc.*, **77**, 1831 (1955).

(5) F. E. Hahn, R. L. O'Brien, J. Ciak, J. L. Allison, and J. G. Olenick, *Military Med., Suppl.* **131**, 1071 (1966); also J. Ciak and F. E. Hahn, *Science*, **156**, 655 (1967).

(6) We do not have the results of similar variations of side-chain unsaturation in quinacrine as yet, but work is being pursued in this area.

SCHEME I



extent in the *cis* configuration. The acetylenic compound **4**, where the four C atoms are present in a straight line, exhibited greater curative activity in mice than chloroquine and no toxicity up to 640 mg/kg.

As soon as the 1-methyl group is eliminated, as in **1**–**3**, the structure–activity picture is considerably modified again.

The data presented in this paper are not adequate to justify concrete conclusions. They only indicate that the relationship of the geometry of the side chain in chloroquine analogs to biological activity might be more complex than the consideration of the additive bond distance between the two N atoms alone. Configurational differences seem to be important. Chemical involvements at the DNA site, at the time of adjustment or afterwards, cannot be ruled out. Besides, several other modes of antimalarial action of chloroquine and its unsaturated analogs might also be considered. It should be of interest to study the metabolic products of these unsaturated compounds to see how far the chemical action at the centers of unsaturation is (or is not) involved in modifying the antimalarial activity.

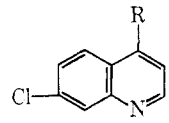
Biological Activity.—These compounds were tested for their antimalarial activity against *Plasmodium berghei* in mice. The screening was carried out by Dr. L. Rane of the University of Miami, Miami, Fla. The screening procedure has been described.[7]

All six compounds showed a wide range of active, curative, and toxic properties as compared to chloroquine (Table II). Compound **1**, with an acetylenic side chain without the 1-methyl group, was only slightly active at 320 and 640 mg/kg, while **4**, with 1-methyl group, was active at 20 mg/kg and cured two, five, and five mice at 160, 320, and 640 mg/kg, respectively. Compound **2**, *cis* ethylenic without 1-methyl, was toxic while **5**, *cis* ethylenic with 1-methyl, cured five mice at 160 mg/kg and became toxic as the dose was increased. Compound **3**, *trans* ethylenic without 1-methyl, was curative without showing any toxicity up to 640 mg/kg, while **6**, *trans* ethylenic with 1-methyl, was highly toxic, killing all five mice at 320 mg/kg. Chloroquine under the same screening conditions was more toxic than any of the six experimental compounds.

Similarly, there are very significant differences in activity and toxicity when we compare **1**, **2**, and **3**, or **4**, **5**, and **6**.

(7) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).

TABLE I



No.	R	Yield, %	Mp, °C	Crystn solvent	Formula[a]
1	$NHCH_2C(H)\equiv C(H)CH_2N(C_2H_5)_2$	63.0	162–163.5	EtOAc	$C_{17}H_{20}ClN_3$
2	$NHCH_2C(H)=C(H)CH_2N(C_2H_5)_2$	8.0	138.5–140	EtOAc	$C_{17}H_{22}ClN_3$
3	$NHCH_2C(CH_3)=C(H)CH_2N(C_2H_5)_2$	32.0	121–122.5	EtOAc	$C_{17}H_{22}ClN_3$
4	$NHCH(CH_3)C(H)\equiv C(H)CH_2N(C_2H_5)_2$	44.0	105–106	Cyclohexane	$C_{18}H_{22}ClN_3$
5	$NHCH(CH_3)C(H)=C(H)CH_2N(C_2H_5)_2$	72.0	145–146.5	Cyclohexane	$C_{18}H_{24}ClN_3$
6	$NHCH(CH_3)C(H)=C(H)CH_2N(C_2H_5)_2$	46.0	90–91.5	Cyclohexane	$C_{18}H_{24}ClN_3$

[a] All compounds were analyzed for C, H, N.

TABLE II

	Antimalarial activity[b]					Antimalarial activity[b]				
No.	D	C	TD	Incr in MST	Remarks[c]	No.	D	C	TD	Incr in MST	Remarks[c]
1[a]	20	0	0	0.8			320	5	0		Curative
	40	0	0	3.0			640	5	0		Curative
	80	0	0	3.6		5	20	0	0	9.7	Active
	160	0	0	5.2			40	3	0	19.5	Curative
	320	0	0	6.4	Active		80	5	0		Curative
	640	0	0	9.6	Active		160	5	0		Curative
2	20	0	0	3.5			320	4	1		Curative, toxic
	40	0	0	5.5			640	3	2		Curative, toxic
	80	0	0	6.3	Active	6	10	0	0	7.7	Active
	160	0	0	11.3	Active		20	2	0	14.2	Curative
	320	0	0	13.3	Active		40	2	0	15.5	Curative
	640	1	4		Curative, toxic		80	2	0	21.5	Curative
3	20	0	0	5.1			160	4	1		Curative, toxic
	40	0	0	9.5	Active		320	0	5		Toxic
	80	0	0	10.7	Active	Chloroquine	20	0	0	6.5	Active
	160	2	0	14.7	Curative		40	0	0	7.5	Active
	320	3	0	16.2	Curative		80	0	1	8.9	Active, toxic
	640	3	0	19.7	Curative		160	0	3	15.9	Active, toxic
4	20	0	0	8.5	Active		320	0	5		Toxic
	40	0	0	11.9	Active						
	80	0	0	13.5	Active						
	160	2	0	16.2	Curative						

[a] Numbers refer to those in Table I. [b] D, dose in mg/kg; C, cures; MST, mean survival time of the treated mice; TD, toxic deaths when mice die 2–5 days postinfection, attributed to drug toxicity. [c] A compound is active if the increase in MST of the treated mice exceeds 6.1–6.5 days (the MST of the control group of mice). A compound is curative if one or more mice live for 60 days or more postinfection.

Experimental Section

All melting points were taken in open capillary tubes in a Thomas–Hoover Unimelt and are uncorrected.

4-Diethylamino-*cis*-2-butenylamine-N-phthalimide (VII, R = H).—A mixture of 4-diethylamino-2-butynylamine-N-phthalimide (V, R = H) (3.0 g, 0.011 mol), Lindlar catalyst (0.2 g), and 50 ml of EtOAc was hydrogenated at 22° and atmospheric pressure. One equivalent of H_2 was absorbed in 2.5 hr. The mixture was filtered through Celite, the filtrate was concentrated to an oil, and the oil was distilled at 110–115° (0.007 mm) in the Kugelrohr apparatus to give 2.8 g (93.2%) of the product. *Anal.* ($C_{16}H_{20}N_2O_2$) C, H, N.

4-Diethylamino-*cis*-2-butenylamine (IX, R = H).—A mixture of the phthalimide VII (R = H) (14.5 g, 0.053 mol), 97% hydrazine (1.75 g, 0.053 mol), and 60 ml of EtOH was refluxed for 5 hr. The mixture was filtered, and the filtrate was concentrated to an oil and distilled to give 3.7 g (48.0%) of IX, bp 165–167° (760 mm), $n^{19}D$ 1.4674. Glpc on a 20% Carbowax–firebrick column, oven temperature 160°, showed the material to be 98.0% *cis* and 2.0% *trans* isomer. The product was used as such for its reaction with 4,7-dichloroquinoline.

4-Diethylamino-*trans*-2-butenylamine (VIII, R = H).—4-Diethylamino-2-butynylamine (VI, R = H) (20.0 g, 0.143 mol) was added dropwise to a stirred solution of Na (13.8 g, 0.60 g-atom) in 500 ml of liquid NH_3 in 0.5 hr. The reaction was allowed to stir for an additional period of 2 hr. NH_3 was allowed to evaporate off. Some H_2O was added to the residue and the mixture was extracted with Et_2O (three 100-ml portions). The Et_2O extracts were worked up in the usual manner to give an oil which distilled at 80° (10 mm), yield 11.0 g (55.0%). The amine was hygroscopic. It proved to be 99.0% *trans* with a trace of *cis* isomer by glpc on a 20% Carbowax–firebrick column at oven temperature of 160°. It was used as such for its reaction with 4,7-dichloroquinoline.

3-Bromo-1-butyne (III, R = CH_3) was prepared by the method of Rogers and Panish[8] in 31.0% yield, bp 44–48° (0.5 mm), $n^{20}D$ 1.4738.

1-Methyl-2-propynylamine-N-phthalimide (IV, R = CH_3).—3-Bromo-1-butyne (27.0 g, 0.15 mol) was slowly added to a mixture of potassium phthalimide (27.0 g, 0.15 mol) in 100 ml of dry DMF maintained at 70°. The mixture was stirred at this temperature for 12 hr and then cooled and poured into 5 vol of H_2O. The precipitated solid was filtered off, washed (H_2O), and crystallized (MeOH–H_2O) to give 20.0 g (65.0%) of product, mp 111–112.5°. *Anal.* ($C_{12}H_9NO_2$) C, H, N.

4-Diethylamino-1-methyl-2-butynylamine-N-phthalimide (V, R = CH_3).—A mixture of IV (R = CH_3) (60.0 g, 0.30 mol), paraformaldehyde (10.8 g, 0.36 mol), Et_2NH (24.0 g, 0.37 mol), and 50 ml of dioxane was refluxed for 4 hr. It was cooled and diluted with 7 vol of H_2O. The mixture was acidified to pH 4 and extracted with Et_2O (two 200-ml portions). The aqueous layer was neutralized with K_2CO_3 to pH 10–11 and extracted with CH_2Cl_2 (three 250-ml portions). The CH_2Cl_2 extracts were dried ($CaCl_2$) and concentrated to an oil which was distilled at 110–115° (0.2 mm) to give 66.0 g (77.0%) of the product. *Anal.* ($C_{17}H_{20}N_2O_2$) C, H, N.

4-Diethylamino-1-methyl-2-butynylamine (VI, R = CH_3) Dihydrochloride.—A mixture of V (R = CH_3) (75.0 g, 0.264 mol), hydrazine hydrate (15.3 g, 0.27 mol), and 350 ml of EtOH was refluxed for 4 hr. It was cooled, acidified with concentrated HCl, and filtered and the solid was washed with 95.0% EtOH (three 100-ml portions). The filtrate was concentrated to a solid, dissolved in a minimum amount of H_2O, basified with 50% KOH solution with cooling, and extracted with Et_2O (three 100-ml portions). The combined Et_2O extracts were dried (K_2CO_3), concentrated, and distilled at 90–92° (10 mm) to give 24.5 g (60.0%) of the amine. A portion was converted to a dihydrochloride salt which was crystallized from *i*-PrOH, mp 167–168.5°. *Anal.* ($C_9H_{20}N_2Cl_2$) N.

4-Diethylamino-1-methyl-*trans*-2-butenylamine (VIII, R = CH_3).—Compound VI (R = CH_3) (7.0 g, 0.045 mol) was reduced with Na–liquid NH_3 in 74.0% yield in the same manner as described for VIII (R = H); bp 74° (8 mm). Glpc on a 20% Carbowax–firebrick column showed it to be 100% pure *trans* isomer.

Reaction of the Unsaturated Amines with 4,7-Dichloroquinoline (1–4, 6).—The same general procedure was followed for this reaction which consisted of heating a mixture of 4,7-dichloroquinoline (0.04 mol) and the unsaturated amine (0.05 mol) in 40 ml of phenol at 140–145° for about 4 hr. The mixture was cooled, poured into 15% NaOH solution, and extracted (CH_2Cl_2 or Et_2O), and the extracts were dried (K_2CO_3) and evaporated to give an oil which usually solidified on cooling, standing, or scratching. The solid was then crystallized from a suitable solvent (see Table I). In one case (**6**), the oil was distilled at 155–160° (0.2 mm) in a Kugelrohr apparatus. The distillate solidified on standing.

7-Chloro-4-(4-diethylamino-1-methyl-*cis*-2-butenylamino)-quinoline (5).—A mixture of 7-chloro-4-(4-diethylamino-1-methyl-2-butynylamino)quinoline (**4**) (3.3 g, 0.0105 mol), Lindlar catalyst (0.3 g), and 75 ml of EtOAc was hydrogenated at room temperature and atmospheric pressure. H_2 uptake was complete in 3 hr. The catalyst was filtered through Celite and the filtrate was evaporated to dryness to give a white solid which was crystallized from cyclohexane (see Table I).

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(8) M. T. Rogers and M. B. Panish, *J. Amer. Chem. Soc.*, 77, 3684 (1955).

3,5-Dinitrosalicylic Acid (5-Nitrofurfurylidene)hydrazide, a Potent New Preventive of Histomoniasis in Turkeys

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A series of substituted benzoic acid (5-nitrofurfurylidene)hydrazides has been prepared and screened extensively for antibacterial and antiprotozoal activity. One of these compounds, 3,5-dinitrosalicylic acid (5-nitrofurfurylidene)hydrazide (I), has shown outstanding antihistomonal activity in poultry. The related compounds that have been synthesized and their test results in preventing blackhead disease in turkeys are presented. Possible reasons for the high degree of activity and specificity of I are discussed.

In recent years, a considerable number of reports have been published on the biological properties of nitrofurans, especially their antibacterial activity. During a search for novel nitrofurans which might have antibacterial or antiprotozoal properties, it was noted that few benzoic acid (5-nitrofurfurylidene)hydrazide derivatives had been reported. Numerous nitrofurans of this type were subsequently prepared in our laboratory and were screened against the blackhead parasite *Histomonas meleagridis* as well as other organisms. One of these compounds, 3,5-dinitrosalicylic acid (5-nitrofurfurylidene)hydrazide (I) was found to be exceptionally effective in preventing blackhead disease in poultry. All of the related compounds in Table I (II–XXI) that were prepared had little or no activity against blackhead. Each of these 21 compounds was screened by administering the compound in the feed to turkeys at 0.05% or down to the least effective level. These compounds were prepared, with the exception of XVI, by one of three general methods: A, treatment of the substituted benzoic acid hydrazide with 5-nitro-2-furanmethanediol diacetate and mineral acid catalyst; B, treatment of the substituted benzoic acid hydrazide with 5-nitro-2-furaldehyde; C, treatment of the substituted benzoyl chloride with the appropriate hydrazone.