Antimalarial Agents IX

3-Alkylquinolones as Potential Repository Drugs

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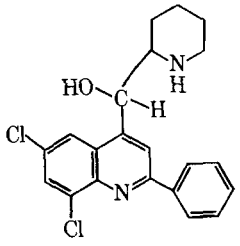
As a possible answer to the need for antimalarials with more prolonged activity as well as for drug resistant malaria, several compounds related structurally to endochin (II) have been synthesized. They failed to pass preliminary pharmacological requirements.

ALTHOUGH the 4-aminoquinoline antimalarial agents, chloroquine and amodiaquine, are generally effective suppressive agents in areas of endemic malaria, they must be readministered every few weeks to maintain therapeutic effectiveness (1). An objective of the present study is the synthesis of certain substituted quinolines which might show prolonged antimalarial activity. Should such agents be found, it is hoped that prolonged activity might also contribute effectiveness against the recent serious threat posed by resistant falciparum malaria (2).

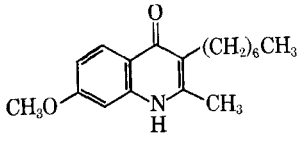
DISCUSSION

Compound I (SN-10,275) (3) once gave promise of possessing prolonged activity because 1 to 5% of the drug was still present in the plasma as long as 3 months following the last dose (4). However, photosensitivity manifest by severe itching prohibits administration to humans.



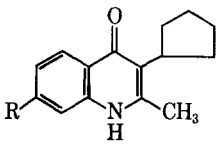
I

SN-10,275



II

SN-13,421 (Endochin)

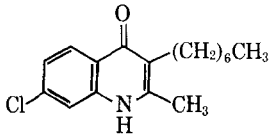


R

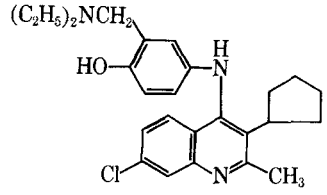
a, Cl

b, CH_3O

III



IV



V

Endochin (II) (SN-13,421) has shown prophylactic activity against *Plasmodium cathemerium* in the canary, *P. gallinaceium* in the chick, and *P. lophurae* in the turkey (5). In view of the lipophilic alkyl grouping in position 3 of the quinoline nucleus, it was believed that endochin or its variants might be held in the tissues and gradually released to contribute prolonged antimalarial activity (6). Such structures as III*a* and IV were proposed since they contain structural features of both endochin and I. Particularly, a substituent at the 2 position would not allow the deactivating 2-hydroxylation which occurs upon administration of quinine (7). Since quinoline antimalarials containing a basic side chain at position 4 generally possess greater potency than the parent compound, structure V was desired for comparative biological evaluation with types III and IV.

Compounds of type III were synthesized by the Conrad-Limpach method (8) as outlined in Scheme I.

Ethyl α-cyclopentylacetoacetate (VI) was prepared from bromocyclopentane and ethyl acetoacetate according to the method of Rydon (9). Two methods were employed for the formation of the anilinocrotonates (VII). It was desirable to use conditions that were as mild as possible to avoid any formation of an anilide (IX) which could result at higher temperatures through a Knorr-type condensation (10). In method *A*, a *m*-substituted aniline and ethyl α-cyclopentylacetoacetate (VI)

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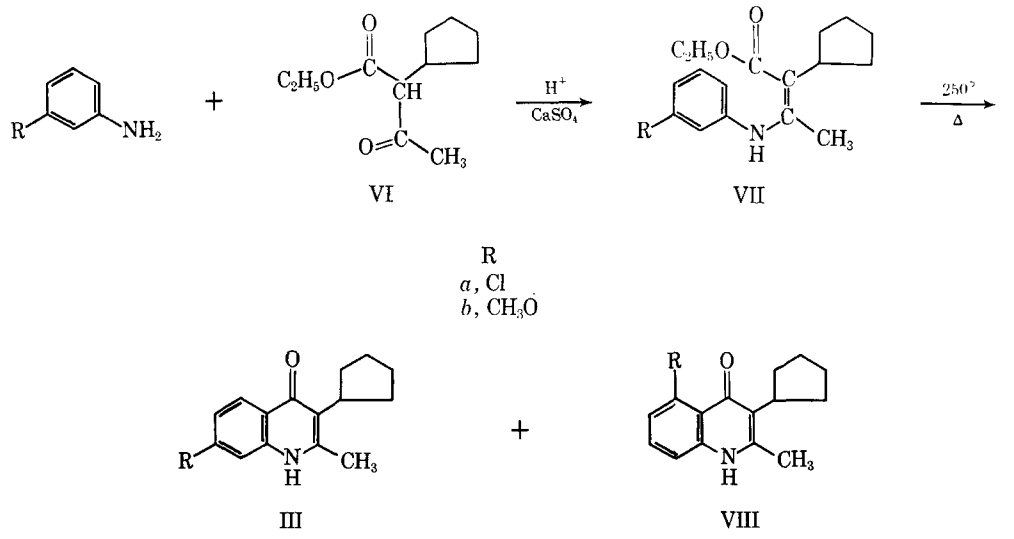
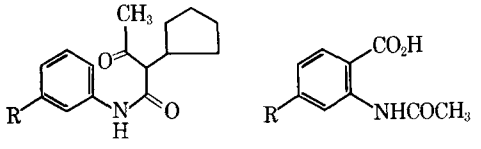


Scheme I



IX X

were heated in ethanol with an excess of anhydrous calcium sulfate and a catalytic amount of the appropriate aniline hydrochloride, according to an early procedure of Hauser and Reynolds (11). Anil formation has been shown to be acid catalyzed (12).

The second method (method *B*) involved the removal of water by azeotropic distillation and gave an increase in yield without apparent adverse reactions (13).

With the exception of ethyl β-(*m*-chloroanilino)-α-cyclopentylcrotonate, the anils were cyclized directly after removal of excess starting materials when it was found that ring closure occurred during attempted distillation. Cyclizations were carried out by addition of the anilinocrotonates (VII) to a refluxing mixture of diphenyl ether and biphenyl, according to the procedure of Hauser and Reynolds (14).

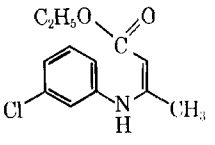
Infrared spectra of the products determined in potassium bromide disks show that the 7-substituted-3-cyclopentyl-2-methyl-4-quinolinols exist in the quinolone (III) form. Absorption peaks appear at 3330 cm.$^{-1}$ characteristic of NH stretching and at 1637 cm.$^{-1}$ characteristic of the lactam carbonyl stretching frequency (15).

A major difficulty with the Conrad-Limpach synthesis, as well as with the Skraup synthesis, is the formation of mixtures of 5- and 7-substituted quinolines when *m*-substituted anilines are used (16). Fortunately, only 3-cyclopentyl-7-methoxy-2-methyl-4-quinolone (III*b*) was isolated when ethyl α - cyclopentyl - β - *m* - methoxyanilinocrotonate (VII*b*) was cyclized. However, in the case of the reaction as applied to *m*-chloroaniline a small amount of solid, m.p. 274–276°, was isolated from the filtrate after removal of 7-chloro-3-cyclopentyl-2-methyl-4-quinolone (III*a*); it is undoubtedly the 5-chloro isomer (VIII). A comparison of its infrared spectrum with that of the 7-chloro isomer (III*a*) showed the two spectra to be very similar except in the region characteristic of aromatic substitution. The spectrum of the lower melting 5-chloro isomer (VIII) contained a broad double peak at 790–810 cm.$^{-1}$ characteristic of three adjacent free hydrogens, while this peak was missing from the spectrum of the 7-chloro isomer (17).

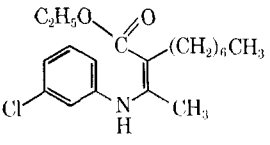
The structures of the 7-substituted quinolones (III*a* and *b*) were proved by oxidation to the appropriate *N*-acetyl-4-substituted anthranilic acids (X*a* and *b*) with alkaline potassium permanganate in the manner of Elderfield and Wright (18). 3-Cyclopentyl-2-methyl-7-methoxy-4-quinolone (III*b*) gave on oxidation *N*-acetyl-4-methoxyanthranilic acid (X*b*), m.p. 197–199°, corresponding to that reported by Friedlander, Bruckner, and Deutsch (19). The melting point of the isomeric *N*-acetyl-6-methoxyanthranilic acid is 176° (19).

Inasmuch as the melting points of the *N*-acetyl derivatives of both 4-chloroanthranilic acid and 6-chloroanthranilic acid are nearly identical (214 and 215°), it was necessary to hydrolyze the amide (X*a*) to the free base. The melting point of the 4-chloroanthranilic acid thus obtained (m.p. 235–238° dec.) corresponded to that reported by Cohn (20). He also reported the melting point of 6-chloroanthranilic acid to be 146–147° dec. (21).

The 7-chloro analog of endochin, 7-chloro-3-heptyl-2-methyl-4-quinolone (IV), was synthesized according to the general procedure of Leonard, Herbrandson, and Van Heynigen (22). The condensation of *m*-chloroaniline and ethyl acetoacetate proceeded smoothly to give ethyl β-*m*-chloro-anilinocrotonate (XI) in 81% yield. XI was alkylated with iodoheptane using sodium hydride in xylene



XI



XII

as the condensing agent. After removal of the solvent and excess starting materials under vacuum, the crude anil (XII) was added directly to a refluxing mixture of diphenyl ether and biphenyl. The yield of 7-chloro-3-heptyl-2-methyl-4-quinolone (IV) was disappointing; only 9% yield based on *m*-anisidine and ethyl acetoacetate was obtained. I.R. suggested a 7-chloro rather than a 5-chloro substituent.

In an attempt to study the effect of a basic side chain upon the antimalarial activity of compounds of the endochin type, it was decided to attach the 4-amino-α-diethylamino-*o*-cresol side chain to 7-chloro - 3 - cyclopentyl - 2 - methyl - 4 - quinolone (III*a*). Treatment of III*a* in methylene chloride with phosphoryl chloride gave 3-cyclopentyl-4,7-dichloro-2-methylquinoline in high yield. This intermediate was condensed with 4-amino-α-diethylamino-*o*-cresol dihydrochloride in the manner of Burckhalter and co-workers (23). 4-(7-Chloro-3-cyclopentyl - 2 - methyl - 4 - quinolylamino) - α-diethylamino-*o*-cresol dihydrochloride (V) was obtained in 54% yield.

PHARMACOLOGICAL RESULTS

Compounds III*a*, III*b*, IV, and V were evaluated as antimalarial agents by Dr. Paul E. Thompson, Research Laboratories, Parke, Davis and Co., Ann Arbor, Mich. III*a* and III*b* were examined for suppressive activity against *P. lophurae* in chicks, according to the method of Thompson (24). Both substances, fed in diet concentrations of 0.05 and 0.2%, were found to be inactive.

IV and V dihydrochloride were tested by Thompson against *P. berghei* in mice by subcutaneous injection. The former in a single subcutaneous dose of 400 mg./Kg. and the latter in twice daily doses of 5 mg./Kg. for 4 days failed to prevent the development of malarial infections. Since quinine is active at 25 mg./Kg., V is less than 5 times as active as quinine.

None of the compounds appear to offer promise as repository agents.

EXPERIMENTAL

Ethyl β - m - Chloroanilino - α - cyclopentylcrotonate (VII*a*).—*Method A.*—A modification of the general procedure of Hauser and Reynolds was used (11). To a mixture of 12.8 Gm. (0.1 mole) of *m*-chloroaniline and 18.2 Gm. (0.1 mole) of ethyl α - cyclopentylacetoacetate (9) in 20 ml. of absolute ethanol, 27 Gm. of anhydrous calcium sulfate and a catalytic amount (12) (0.01 Gm.) of *m*-chloroaniline hydrochloride were added. The mixture was stirred and heated for 4 hr. at 70–80°. The calcium sulfate was removed by filtration and the filtrate was concentrated and distilled under vacuum, giving 15 Gm. (55% yield) of the anilinocrotonate (VII*a*), b.p. 137° (0.2 mm.).

Anal.—Calcd. for $C_{17}H_{22}ClNO_2$: C, 66.33; H, 7.20. Found: C, 66.11; H, 7.29.

Method B.—The improved method of Hauser and Reynolds was employed (13). A mixture of 0.1 mole of *m*-chloroaniline, and 0.1 mole of ethyl α-cyclopentylacetoacetate (9) and 50 ml. of benzene was placed in a flask fitted with a Dean-Stark water separator and condenser. After addition of 0.5 ml. of acetic acid, the mixture was heated to reflux until water no longer separated. After 72 hr., 1.2 ml. of water had collected. The mixture was concentrated and excess *m*-chloroaniline and ester were distilled under vacuum, giving 22 Gm. (72% yield) of crude product. The VII*a* was pure enough to use in the next step.

7 - Chloro - 3 - cyclopentyl - 2 - methyl - 4 - quinolone (III*a*).—A solution of 15 Gm. (0.049 mole of ethyl β - *m* - chloroanilino - α - cyclopentylcrotonate (VII*a*) in 30 ml. of diphenyl ether was added dropwise to 150 ml. of a refluxing mixture of diphenyl ether and biphenyl, according to the procedure of Hauser and Reynolds (14). After the mixture was cooled, the gray solid which separated was collected on a filter, washed with petroleum ether (60–70°), and dried to yield 7 Gm. of crude III*a*, m.p. 334–336°. Recrystallization from dilute alcohol gave 5 Gm. (39% yield) of III*a*, m.p. 362–365° dec. $\nu_{max.}^{KBr}$ in cm.$^{-1}$ 3300 (NH), 1639 (lactam C=O), 780 (2-adj. free H's).

Anal.—Calcd. for $C_{15}H_{16}ClNO$: C, 68.83; H, 6.16. Found: C, 68.79; H, 6.28.

The filtrate from the reaction just described was diluted with 300 ml. of petroleum ether (60–70°) and the solid which separated was collected on a filter. After recrystallization from dilute alcohol, 1.3 Gm. of a white solid (VIII) was obtained, m.p. 276–278° corrected. Infrared: $\nu_{max.}^{KBr}$ in cm.$^{-1}$, 3300 (NH), 1637 (lactam C=O), 788, 812 (3-adj. free H's).

Anal.—Calcd. for $C_{15}H_{16}ClNO$: C, 68.83; H, 6.16. Found: C, 69.03; H, 6.21.

Proof of Structure III*a*: Its Oxidation to N-Acetyl-4-chloroanthranilic Acid (X*a*).—A suspension of 6 Gm. (0.023 mole) of III*a* in 1500 ml. of water made alkaline with 2.5 Gm. of potassium hydroxide was oxidized with 40 Gm. of potassium permanganate according to the procedure of Elderfield and Wright (18). After acidification of the concentrate with concentrated hydrochloric acid, 2 Gm. of *N*-acetyl-4-chloroanthranilic acid (X*a*) was obtained, m.p. 213.5–215.5° corrected. [Lit. (20) m.p. 215°.]

Hydrolysis of the amide (X*a*) in 10% hydrochloric acid, followed by neutralization with sodium bicarbonate, gave 0.2 Gm. of 4-chloroanthranilic acid, m.p. 234.5–237.5° corrected. [Lit. (20) m.p 235°.]

3 - Cyclopentyl - 2 - methyl - 7 - methoxy - 4-quinolone (III*b*).—A mixture of 12.3 Gm. (0.1 mole) of *m*-anisidine and 18.2 Gm. (0.1 mole) of ethyl α-cyclopentylacetoacetate (9) was condensed according to the method of Hauser and Reynolds (11). After distillation under vacuum, 14.5 Gm. (47% yield) of ethyl α-cyclopentyl-β-*m*-methoxyanilinocrotonate (VII*b*) was obtained, b.p. 150° (0.4 mm.), n_D^{27} 1.5252.

A solution of 14 Gm. (0.046 mole) of the foregoing anilinocrotonate (VII*b*) in 30 ml. of diphenyl ether was added dropwise to 250 ml. of a refluxing

mixture of diphenyl ether and biphenyl. The mixture was heated for an additional 30 min., cooled, and the light tan product collected on a filter, washed with petroleum ether (60–70°), and dried giving 5 Gm. (42% yield) of *IIIb*, m.p. 310–312° dec. Recrystallized from 75% ethanol, it melted at 312–313° dec.

Anal.—Calcd. for $C_{16}H_{19}NO_2$: C, 74.68; H, 7.44. Found: C, 74.64; H, 7.49.

Proof of Structure IIIb: Its Oxidation to N-Acetyl-4-methoxyanthranilic Acid (Xb).—A suspension of 6 Gm. (0.023 mole) of 3-cyclopentyl-2-methyl-7-methoxy-4-quinolone (*IIIb*) in 1500 ml. of water containing 2.5 Gm. of potassium hydroxide was oxidized with potassium permanganate according to the procedure of Elderfield and Wright (18). The mixture was filtered free of manganese dioxide, concentrated to one-third of its volume, and acidified with concentrated hydrochloric acid, giving 1.5 Gm. of crude *N*-acetyl-4-methoxyanthranilic acid (*Xb*). Recrystallization first from water and then from methanol gave 1 Gm. of white needles, m.p. 196.5–198.5° corrected. [Lit. (19) m.p. 197–199°.]

Ethyl β - m - Chloroanilinocrotonate (XI).—A mixture of 25.6 Gm. (0.2 mole) of *m*-chloroaniline and 26 Gm. (0.2 mole) of ethyl acetoacetate was condensed according to the general procedure of Hauser and Reynolds (11). Vacuum distillation gave 39 Gm. (81% yield) of XI, b.p. 139–141° (1.2 mm.). [Lit. (12) b.p. 145–148° (1.5 mm.).]

7 - Chloro - 3 - heptyl - 2 - methyl - 4 - quinolone (IV).—A solution of 39 Gm. (0.16 mole) of ethyl β - *m* - chloroanilinocrotonate (11) (XI) in 100 ml. of dry xylene was alkylated with 37 Gm. (0.113 mole) of 1-iodoheptane according to the procedure of Leonard, Herbrandson, and Van Heynigen (22). After filtration, and removal of solvent, the crude oily residue (40 Gm.) was cyclized directly.

To a refluxing solution of 500 ml. of diphenyl ether–biphenyl mixture, 40 Gm. of crude ethyl β - *m* - chloroanilino - α - heptylcrotonate (XII) was added dropwise. After the addition was complete, heating was continued for 20 min. and the mixture chilled. White plates were collected on a filter and washed with petroleum ether (60–70°). Recrystallization from 95% ethanol gave 4.3 Gm. (9% yield) of IV, m.p. 260–262°. $\nu_{max.}^{KBr}$ in cm.$^{-1}$ 3300 (NH), 1637 (lactam C=O), 775 (2 adj. free H's).

Anal.—Calcd. for $C_{17}H_{22}ClNO$: C, 69.97; H, 7.60. Found: C, 69.94; H, 7.44.

3 - Cyclopentyl - 4,7 - dichloro - 2 - methylquinoline.—A mixture of 25 Gm. (0.095 mole) of 7 - chloro - 3 - cyclopentyl - 2 - methyl - 4 - quinolone (*IIIa*) and 75 Gm. (0.5 mole) of phosphoryl chloride was heated on a steam bath for 2 hr. Excess phosphoryl chloride was removed *in vacuo*, and the residue taken up into 200 ml. of methylene chloride. This solution was allowed to flow slowly into a vigorously stirred mixture of ice and water. The two-phase solution was neutralized with concentrated ammonium hydroxide and the resulting mixture extracted with 100 ml. of methylene chloride. The extracts were washed with water and dried over anhydrous potassium carbonate. The dried solution was filtered through a layer of charcoal and diatomaceous earth,[1] and concentrated to dryness. Recrystallization of the residue from methanol gave 26 Gm. (98% yield) of product, m.p. 86–88°.

Anal.—Calcd. for $C_{15}H_{15}Cl_2N$: C, 64.30; H, 5.40. Found: C, 64.06; H, 5.44.

4 - (7 - Chloro - 3 - cyclopentyl - 2 - methyl - 4-quinolylamino) - α - diethylamino - o - cresol (V) Dihydrochloride.—A mixture of 5.2 Gm. (0.019 mole) of 3-cyclopentyl-4,7-dichloro-2-methylquinoline, 4.8 Gm. (0.019 mole) of 4-amino-α-diethylamino-*o*-cresol dihydrochloride (23), and 30 ml. of ethanol was refluxed for 24 hr. on a steam bath. The mixture was cooled, and the solid was collected on a filter giving 2.4 Gm. of orange crystals, m.p. 257–259° corrected. The filtrate was refluxed for an additional 24 hr., cooled, and the product again collected on a filter. After repetition of this procedure a second time, a total of 7.7 Gm. of crude product was obtained. Recrystallization from methanol–acetone gave 5.1 Gm. (54% yield) of V dihydrochloride as orange crystals, m.p. 252–253°. A sample for analysis was recrystallized twice from absolute alcohol, m.p. 252–254° dec.

Anal.—Calcd. for $C_{26}H_{32}ClN_3$ 0.2 HCl: C, 61.12; H, 6.31. Found: C, 60.93; H, 6.39.

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[1] Marketed as Celite by the Johns-Manville Corp., New York, N. Y.