Chemical and Biological Studies on 1,2-Dihydro-*s*-triazines XVIII: Synthesis of 1-(5,6- and 5,7-Dichloro-2-naphthyl) Derivatives and Related Compounds as Candidate Antimalarial and Antitumor Agents

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Abstract □ 4,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazines with 5,6- and 5,7-dichloro-2-naphthyl groups at position 1 were synthesized and evaluated for biological activity. The 5,6-dichloro compound caused moderate inhibition of *Streptococcus faecium* and of human epidermal carcinoma (KB cells) *in vitro*, as well as a moderate increase in mean survival of mice bearing P1534 leukemia in the ascitic form. The 5,7-dichloro analog was active against *Plasmodium gallinaceum* in chicks.

Keyphrases □ 4,6-Diamino-1,2-dihydro-*s*-triazines, dichloronaphthyl substituted—synthesis, screened as potential antimalarial and antitumor agents □ 1,2-Dihydro-*s*-triazines, 5,6- and 5,7-dichloro-2-naphthyl derivatives—synthesis, screened as potential antimalarial and antitumor agents □ Antimalarial agents, potential—synthesis and screening of 4,6-diamino-1,2-dihydro-*s*-triazines with dichloronaphthyl substitution □ Antitumor agents, potential—synthesis and screening of 4,6-diamino-1,2-dihydro-*s*-triazines with dichloronaphthyl substitution

The availability in this laboratory of several heteronuclear dichloro derivatives of 2-naphthylamine (1) in connection with another investigation (2) afforded an unusual opportunity for the synthesis and biological evaluation of some hitherto inaccessible 4,6-diamino-1,2-dihydro-*s*-triazine antifolates with a halogenated 2-naphthyl moiety at position 1. These compounds, as well as the previously reported 2-naphthyl (3, 4) and 6-chloro-2-naphthyl (5) derivatives, were viewed with interest because of the high level of antifolate activity displayed in various test systems by other aryldihydrotriazines, including especially the phenyl and 1-naphthyl types (6).

The present paper describes the synthesis of 4,6-diamino-1-(5,6-dichloro-2-naphthyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine hydrochloride (*Ia*·HCl) and its 5,7-dichloro-2-naphthyl analog (*Ib*·HCl). Also described are the corresponding naphthylbiguanide salts (*IIa*·HCl and *IIb*·HCl) and guanidinoquinazoline salts (*IIIa*·HCl and *IIIb*·HCl). Biguanides are known intermediates in the three-component synthesis of dihydrotriazines (7), and guanidinoquinazolines were reported recently to be occasional by-products in this reaction (4, 5).

EXPERIMENTAL[1]

4,6-Diamino-1-(5,6-dichloro-2-naphthyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine Hydrochloride (*Ia*·HCl) (Standard Three-Component Synthesis)—A mixture of 10.7 g. (0.043 mole) of 5,6-dichloro-2-naphthylamine hydrochloride (1), 4.51 g. (0.055 mole) of cyanoguanidine, 15 ml. of acetone, and 20 ml. of absolute ethanol was stirred under reflux for 3 hr., allowed to stand at room temperature for 18 hr., and filtered. The solid was washed with acetone (3 × 10 ml.) and dried, yielding 9.57 g. (60%). Two recrystallizations from water with the aid of decolorizing carbon gave analytically pure colorless needles, m.p. 233–235°.

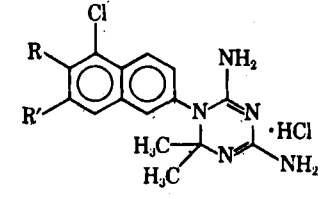
Anal.—Calc. for $C_{15}H_{15}Cl_2N_5 \cdot HCl$: C, 48.34; H, 4.33; Cl, 28.54; N, 18.79. Found: C, 48.46; H, 4.11; Cl, 28.49; N, 18.79.

4,6-Diamino-1-(5,7-dichloro-2-naphthyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine Hydrochloride (*Ib*·HCl) (Two-Component Synthesis)—A mixture of 11.3 g. (0.03 mole) of biguanide *IIb*·HCl, 0.25 ml. of 12 *N* HCl, 50 ml. of acetone, and 50 ml. of absolute ethanol was stirred under reflux for 17 hr., cooled, and filtered. The tan solid was washed with acetone and dried, yielding 9.3 g. (73%). The biguanide test with copper ammonium sulfate (7) was negative (no precipitate). Two recrystallizations from 95% ethanol (charcoal) gave analytically pure *Ib*·HCl as off-white needles, m.p. 221.5–224°.

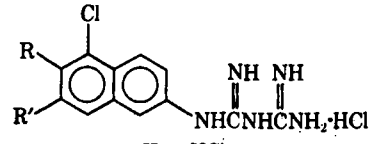
Anal.—Calc. for $C_{15}H_{15}Cl_2N_5 \cdot HCl$: C, 48.34; H, 4.33; Cl, 28.54; N, 18.79. Found: C, 48.43; H, 4.44; Cl, 28.45; N, 18.67.

N^1-(5,6-Dichloro-2-naphthyl)biguanide Hydrochloride (*IIa*·HCl)—A mixture of 10.7 g. (0.0431 mole) of 5,6-dichloro-2-naphthylamine hydrochloride (1), 4.10 g. (0.05 mole) of cyanoguanidine, and 70 ml. of *n*-propanol was stirred under reflux for 3 hr., cooled, and filtered. The tan solid was washed with *n*-propanol (2 × 25 ml.) and dried, yielding 8.18 g. (57%). Three recrystallizations from 95% ethanol (charcoal) afforded colorless needles, m.p. 215.5–216°, with a positive biguanide test (pink solid) (7).

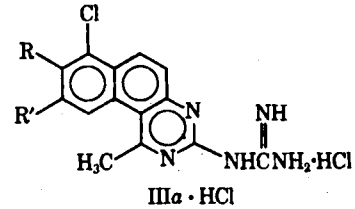
Anal.—Calc. for $C_{12}H_{11}Cl_2N_5 \cdot HCl$: C, 43.33; H, 3.64; Cl, 31.98; N, 21.05. Found: C, 43.23; H, 3.59; Cl, 31.93; N, 20.99.



Ia · HCl
Ib · HCl



IIa · HCl
IIb · HCl



IIIa · HCl
IIIb · HCl

a series: R = Cl, R′ = H
b series: R = H, R′ = Cl

[1] Melting points were taken in Pyrex capillary tubes, using a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.), and are uncorrected. IR spectra were measured in KCl disks with a Perkin-Elmer model 137B recording spectrophotometer. NMR spectra were determined with a Varian A-60 instrument, with trifluoroacetic acid as the solvent and tetramethylsilane as the internal reference.

N^1-(5,7-Dichloro-2-naphthyl)biguanide Hydrochloride (IIb·HCl)—Use of the foregoing procedure with 5,7-dichloro-2-naphthylamine hydrochloride gave a 54% yield of IIb·HCl as an off-white solid. Two recrystallizations from 95% ethanol furnished the analytical sample, m.p. 255–257°.

Anal.—Calc. for $C_{12}H_{11}Cl_2N_5 \cdot HCl$: C, 43.33; H, 3.64; Cl, 31.98; N, 21.05. Found: C, 43.08; H, 3.41; Cl, 32.17; N, 21.04.

7,8-Dichloro-3-guanidino-1-methylbenzo[*f*]quinazoline Hydrochloride (IIIa·HCl)—A solution of 8.88 g. (0.0418 mole) of 5,6-dichloro-2-naphthylamine (1) and 0.5 g. of iodine in 33 ml. of acetone was stirred under reflux for 60 hr., cooled, and evaporated to dryness under reduced pressure. The resulting dark oil was taken up in 50 ml. of 50% ethanol to which were added 6.25 ml. of 12 *N* HCl and 4.75 g. (0.05 mole) of cyanoguanidine. After being stirred under reflux for 1.5 hr., the mixture was refrigerated until crystallization occurred. The solid was filtered, washed with acetone (5 × 5 ml.), and dried, yielding 7.53 g. (51%). Two recrystallizations from 95% ethanol (charcoal) gave colorless crystals, m.p. 312–313° dec. On treatment with copper ammonium sulfate reagent (7), this material gave a light-green precipitate characteristic of guanidinoquinazolines (3).

Anal.—Calc. for $C_{14}H_{11}Cl_2N_5 \cdot HCl$: C, 47.14; H, 3.39; Cl, 29.82; N, 19.64. Found: C, 47.05; H, 3.35; Cl, 29.98; N, 19.61.

7,9-Dichloro-3-guanidino-1-methylbenzo[*f*]quinazoline Hydrochloride (IIIb·HCl) (Modified Three-Component Synthesis)—A mixture of 5.00 g. (0.0235 mole) of 5,7-dichloro-2-naphthylamine (1), 1.97 g. (0.0235 mole) of cyanoguanidine, 0.2 ml. (0.0024 mole) of 12 *N* HCl, 85 ml. of acetone, and 15 ml. of 95% ethanol was stirred at room temperature for 16 hr. and then under reflux for 4 hr. Another 1.75 ml. (0.0211 mole) of 12 *N* HCl was added, refluxing was continued for 70 min., and the precipitated solid was filtered, washed with acetone, and dried, yielding (two crops collected) 4.8 g. (57%); a light-green solid resulted upon treatment with biguanide reagent (3). Two recrystallizations from absolute ethanol (charcoal) gave analytically pure colorless needles, m.p. 337–341° dec.

Anal.—Calc. for $C_{14}H_{11}Cl_2N_5 \cdot HCl$: C, 47.14; H, 3.39; Cl, 29.82; N, 19.63. Found: C, 47.19; H, 3.29; Cl, 29.69; N, 19.56.

RESULTS AND DISCUSSION

5,6-Dichloro-2-naphthylamine hydrochloride was found to give only dihydrotriazine Ia·HCl under the conditions of the standard three-component synthesis (7), with no evidence of the formation of guanidinoquinazoline IIIa·HCl, a comparison sample of which was synthesized *via* the "acetone anil" route described earlier (3). Although IIIa·HCl might have been expected as a by-product on the basis of work with 2-naphthylamine (3), careful NMR spectral analysis of the crude solid filtered from the reaction mixture failed to reveal any of this compound. On the other hand, the standard three-component synthesis with 5,7-dichloro-2-naphthylamine hydrochloride did produce a small amount of guanidinoquinazoline IIIb·HCl, which made it preferable to obtain dihydrotriazine Ib·HCl *via* the two-component synthesis (8) starting from biguanide IIb·HCl. An authentic specimen of IIIb·HCl was synthesized conveniently *via* the recently described (4) modified three-component synthesis, wherein only a limited amount of acid is used at the outset of the reaction. 6-Chloro-2-naphthylamine has been reported (5) to give some guanidinoquinazoline by-product in the standard three-component synthesis, albeit in a much smaller amount than with 2-naphthylamine. Thus, the findings reported here reinforce the previous conclusion that electron-attracting chloro substitution on the aromatic ring diminishes the tendency for guanidinoquinazoline by-product formation (5). However, it appears that 5,7-dichloro substitution is somewhat less effective than 5,6-dichloro substitution in suppressing this side reaction.

Moderate activity was shown against *Streptococcus faecium* (ATCC 8043) (9) by dihydrotriazine Ia·HCl, which gave an ID_{50} value of 0.08 mcg./ml. at a folate level of 0.001 mcg./ml. This may be compared with the ID_{50} value of 0.035 mcg./ml. reported previously by Foley (10) for 4,6-diamino-1-(3,4-dichlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine hydrochloride, which is closely analogous to Ia·HCl in structure.

Activity was also demonstrated for Ia·HCl against human epidermoid carcinoma (KB cells) in culture (11), with an ID_{50} value of 0.5 mcg./ml. observed. However, both the biguanide IIa·HCl (ID_{50} = 0.2 mcg./ml.) and the guanidinoquinazoline IIIa·HCl (ID_{50} = 0.3 mcg./ml.) were at least as active as the dihydrotriazine. Since IIIa·HCl led to only slight inhibition of *S. faecium* (ID_{50} > 10 mcg./ml.), the cytotoxic action of this compound may perhaps be ascribed to some cause other than folate antagonism such as metal chelation. The ability of guanidinoquinazolines to inhibit KB cell growth was noted previously (3, 5), especially with reference to the possibility of error in the bioassay of dihydrotriazines not completely free from guanidinoquinazoline contamination.

Compound Ia·HCl was tested for experimental antitumor activity *in vivo* as described earlier (12), using two transplantable murine leukemias in ascitic form: L-1210 leukemia in BDF/1 hybrid mice and P1534 leukemia in DBA/2 inbred mice. Against L-1210 leukemia, this compound did not significantly prolong the life of the animals at nontoxic doses (up to 40 mg./kg./day × 4). Against P1534 at a dose of 10 mg./kg./day × 4, however, there was a 27% increase in survival relative to the untreated controls. This may be compared to the 30–40% increase reported previously for the 3,4-dichlorophenyl analog against the P1534 tumor (13).

Also assayed for antitumor activity were biguanides IIa·HCl and IIb·HCl and guanidinoquinazoline IIIb·HCl. Although none of these compounds showed any activity against L-1210, biguanide IIa·HCl produced a 32% increase in survival in the P1534 system at a dose of 10 mg./kg./day × 4. Compounds IIb·HCl and IIIb·HCl were inactive.

The compounds were also tested for antimalarial activity against *Plasmodium berghei* in the mouse and P. *gallinaceum* in the chick (14), as well as in the *in vitro* sporozoite suppression assay employing mosquitoes infected with *P. gallinaceum* or *P. cynomolgi* (15). Compound Ib·HCl was active against *P. gallinaceum* in chicks at 80 mg./kg., prolonging the mean survival time from 4.0 to 8.6 days (a 115% increase); at 320 mg./kg., survival was lengthened to 13.6 days (a 240% increase). Compound Ia·HCl caused 100% sporozoite suppression, with partial to complete occurrence of abnormal oocysts, in the blood of infected mosquitoes at an administered concentration of 0.001%. None of the compounds showed significant activity against *P. berghei* in mice.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 11, 1972, from *The Children's Cancer Research Foundation and the Departments of Biological Chemistry and Pathology, Harvard Medical School, Boston, MA 02115*

Accepted for publication September 20, 1972.

Supported in part by Research Contract DA-49-193-3008 from the U. S. Army Research and Development Command, Office of the Surgeon General, and by Research Grant C6516 and Research

Career Development Award K3-CA-22,151 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service. This is paper No. 1084 from the U. S. Army Research Program on Malaria.

The authors are indebted to the following colleagues at The Children's Cancer Research Foundation for their cooperation in obtaining biological activity data: Dr. George E. Foley and Mr. Harold Riley (microbioassay against *Streptococcus faecium* and KB cells in culture), and Dr. Victor M. Rosenoer and Miss Barbara L. Brown (mouse tumor assays). In addition, the authors thank Dr. Edgar A. Steck and Dr. Thomas R. Sweeney, Walter Reed Army Institute of Research, for providing the results of antimalarial assays.

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Vehicle and Route of Administration as Parameters Affecting Operant Behavioral Effects of Δ⁹-Tetrahydrocannabinol

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Abstract □ Four vehicles for Δ⁹-tetrahydrocannabinol were compared after intraperitoneal and subcutaneous administrations, using the disruption of food-reinforced, operant behavior of rats as the test system for cannabinoid activity. Aqueous suspensions based on polyvinylpyrrolidone, polysorbate 80, and a polysorbate 65–sorbitan monolaurate combination all were effective vehicles for intraperitoneal or subcutaneous absorption of the cannabinoid. An olive oil solution was poorly effective. The polyvinylpyrrolidone dispersion appeared to have the most rapid onset of action, while the polysorbate 65–sorbitan monolaurate combination had the longest duration of action.

Keyphrases □ Marijuana—effect of vehicle and route of administration of Δ⁹-tetrahydrocannabinol on operant behavior, rats □ Δ⁹-Tetrahydrocannabinol—effect of vehicle and route of administration on operant behavior, rats □ Cannabinoid activity—studied by operant behavioral effects in rats, different vehicles and routes of administration

The major psychoactive constituent in marijuana (*Cannabis sativa*) is generally accepted to be (−)-Δ⁹-*trans*-tetrahydrocannabinol (I). The elucidation of the structure and synthesis of I has prompted extensive investigation of its pharmacological and behavioral effects in both animals and man (1, 2). However, the extreme aqueous insolubility of I has made parenteral administration difficult. A number of different aqueous suspensions and oil solutions have been tried by various researchers, but no single preparation has found widespread acceptance. At times, conflicting results between studies have been attributed to differences in the drug vehicle or mode of administration.

The present study compared four such vehicles administered *via* two routes of injection to evaluate the drug effect in a test system previously shown to be sensitive to Δ⁹-tetrahydrocannabinol (3–5). The measure chosen was the depression of operant responding of rats for food reinforcement. In this experiment, both the onset and duration of action of I were examined as a function of eight combinations of vehicle and route of injection.

EXPERIMENTAL

Twenty-four male Wistar rats (225–300 g.) were trained to bar-press for food reward on a fixed-ratio-50 (FR50) schedule of reinforcement; the 50th lever-press resulted in the delivery of a 45-mg. Noyes food pellet. The animals were given 60-min. experimental sessions on 5 days each week, with supplementary postsession feeding sufficient to maintain them at 80% of their free-feeding weights. The operant chambers were controlled by solid-state programming equipment. Cumulative recorders and digital counters were used for data collection. Before initial drug sessions, each rat received 4–5 weeks of training until performance stabilized so that there was less than 10% variation in responses per session over three consecutive sessions.

The four preparations of Δ⁹-tetrahydrocannabinol compared in this experiment were: Vehicle A, olive oil solution; Vehicle B, a 1% polysorbate 80[1] dispersion in saline; Vehicle C, a suspension with 1% polysorbate 65[2] and 1% sorbitan monolaurate[3] in 0.9% saline, as described by Moreton and Davis (6); and Vehicle D, a 10% polyvinylpyrrolidone suspension in 0.9% NaCl, prepared according to the procedure of Fenimore and Loy (7). Each vehicle was tested both intraperitoneally and subcutaneously. The dose of I used was 10 mg./kg. given at an injection volume of 1.0 ml./kg. body weight. The injections of I were given at weekly intervals on Wednesday, vehicle alone was given on Tuesday and Thursday, and saline was injected on Monday and Friday. To examine the relative duration of action of I as well as the time of onset, injections were given either immediately prior to the operant session or 1, 2, or 3 hr. before. At least six determinations were made for each vehicle–route–time combination. The drug effect was assessed by comparing the bar-press performance on drug days to performance on the preceding vehicle control session.

RESULTS AND DISCUSSION

Operant responding following the intraperitoneal or subcutaneous injection of the four vehicles alone did not differ from that following saline administration or no injection. In Table I the effects of Δ⁹-tetrahydrocannabinol upon responding are summarized as a function of the route–vehicle–time combinations. The total number of responses during the 1-hr. test session is expressed as a percentage of the responses per hour in the prior vehicle control session. Gen-

[1] Tween 80.
[2] Tween 65.
[3] Arlacel 20.